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Diagnostic and therapeutic use of human maguin protein and nucleic acids for neurodegenerative diseases

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DIAGNOSTIC AND THERAPEUTIC USE OF HUMAN MAGUIN PROTEINS AND NUCLEIC ACIDS FOR NEURODEGENERATIVE DISEASES

The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social and economic burden. Alzheimer's disease is the most common age-related neurodegenerative condition affecting about 10 % of the population over 65 years of age and up to 45 % over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with Alzheimer's disease are senile plaques, composed of amyloid-β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles. AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-192).

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon4 allele of apolipoprotein E (ApoE). Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP), presenilin-1, and presenilin-2, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents.

It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to elucidate the causes and the underlying pathogenesis of neurological diseases and to provide methods, materials, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

Neurons conduct signals in the form of electrical impulses. The communication between the cells of a neuronal network involves several chemical steps, including the production, release, and transport of signal molecules and the recognition of such messengers by a receptor. These steps take place at the anatomical contact of an axon with the dendrites (axonodendritic), the cell body (axonosomatic), or rarely with the axon of another neuron, and also between neurons and cells of muscle- and gland tissue. Trillions of such specialized cell junctions (synapses) in the human brain are crucial for controlling mental activity and learning processes (Tessier-Lavigne and Goodman, *Science* 1996, 274: 1123-1133). A synapse is composed of the presynaptic element, the synaptic cleft with a spacing distance of about 20-30 nm and the postsynaptic component. They are responsible for altering the membrane potential of the postsynaptic neuron or

other effector cells. The quality and intensity of information transferred relies on the number, location, and distribution of synapses. The basis of learning and memory is believed to be due to brain plasticity, i.e. to the plasticity of synapses. The storage and processing of information cause alterations in the structure, chemistry, and strength of synapses and the formation of new synapses (Poirazi and Mel, *Neuron* 2001, 29: 779-796). The postsynaptic density (PSD) is a specialized synaptic signaling assemblage, composed of a specific set of proteins which are assembled together and linked to the cytoplasmic face of the postsynaptic membrane. An important function of the postsynaptic density is the provision of a structural matrix consisting of cytoskeletal and regulatory proteins which localize and accumulate neurotransmitter receptors (e.g. glutamate receptors) and anchor signaling molecules at the postsynaptic membrane (Sheng and Kim, *Current Opinion Neurobiology* 1996, 6: 602-608). Neurotransmitter receptors convert the extracellular chemical signals into intracellular signals. Neurotransmitters are released from the presynaptic membrane into the synaptic cleft via exocytotic processes (vesicle formation). At the postsynaptic membrane they are bound by their specific receptors, resulting in the generation of second messengers. Thus, neurotransmitters function as mediators of nerve impulse transmission across the synapse. The PSD organizes and regulates postsynaptic signal transduction (Kim and Huganir, *Current Opinion Cell Biology* 1999, 11:248-254). To date, several components of the PSD have been identified, among them postsynaptic density (PSD)-95/synapse-associated protein (SAP) 90, the prototypic synaptic scaffolding protein. PSD-95/SAP90 and its isoforms belong to a family of membrane-associated guanylate kinases (MAGUK) (Hirao et al., *Journal of Biological Chemistry* 1998, 273: 21105-21110; Hirao et al., *Journal of Biological Chemistry* 2000, 275: 2966-2972). The members of the MAGUK protein family are multiple PDZ-domain containing proteins which interact with many neuronal adhesion proteins, receptors (e.g. N-methyl-D-aspartate (NMDA)) and ion-channels (e.g. potassium channels) through these domains and mediate their assembling at the PSD (Kim et al., *Neuron* 1996, 17: 103-113).

In this context, a novel neuronal membrane-associated guanylate kinase-interacting protein, denoted MAGUIN, was found by Yao et al. (*Journal of Biological Chemistry* 1999, 274: 11889-11896). Using the PDZ-domain sequence of the neurospecific synaptic scaffolding molecule (S-SCAM) as bait, the authors screened a rat brain yeast two-hybrid library and obtained several positive clones, among them two clones subsequently named rat MAGUIN-1 and rat MAGUIN-2 (GenBank accession numbers AF102853 and AF102854, respectively). A Northern blot analysis of different rat tissues with a rat MAGUIN-1 probe revealed brain specific hybridization signals at 4.4 kDa and 5.4 kDa. MAGUIN proteins have a chimerical molecular structure consisting of several protein modules, an N-terminally located sterile alpha-motif (SAM) (aa 8-75), a PDZ-domain (aa 156-296) and a C-terminally located Pleckstrin-homology (PH)-domain (aa 571-667). The Sterile Alpha Motif (SAM) contains four different domain structures, spanning approximately 70 amino acids generating a compact five-helix bundle with a highly conserved hydrophobic core. This motif was found to be part of a number of types of proteins, including signal transduction proteins, playing a role in mediating protein-protein interactions or DNA binding (Schultz et al., *Protein Science* 1997, 6: 249-253). The term PDZ is named after three proteins (i.e. PSD-95/SAP90, *Drosophila* discs-large tumor suppressor protein (Dlg-A) and the tight junction protein ZO-1), originally identified as proteins sharing the same repeats of about 90 amino acids. These amino acids form a distinctive structure of two α helices and six β sheets which mediate the interaction with the carboxyl termini of various proteins. Often, PDZ-domains of different proteins can heterodimerize with each other. The target proteins are transmembrane receptors, ion channels, or signaling proteins. PDZ-domain binding seems to be important in receptor clustering and in recruiting signal transduction molecules to the plasma membrane. PDZ-domain harboring proteins are for example tyrosine phosphatases and the previously described membrane-associated guanylate kinase-like proteins (Doyle et al., *Cell* 1996, 85: 1067-1076). The Pleckstrin-homology (PH)-domain forms an anti-parallel perpendicular β-sheet sandwich with a succeeding α-helical structure. The β-sheet-loops are important for high affinity binding to specific

phosphatidylinositide phosphates, allowing signaling proteins containing the PH-domain to anchor to membranes, for example as studied for GTP binding proteins, protein kinases and phospholipase C isoforms (Lemmon et al., *Trends Cell Biology* 1997, 7: 237-242).

Rat MAGUIN-1 and rat MAGUIN-2 cover 3099 and 2691 nucleotides of coding sequence, respectively, (GenBank accession numbers AF102853, AF102854) which encode for proteins of 1032 aa amino acids (rat MAGUIN-1) (GenBank accession number aad04568) and 896 amino acids (rat MAGUIN-2) (GenBank accession number aado4567), respectively. Rat MAGUIN-2 lacks the 3' terminal PDZ binding motif of rat MAGUIN-1. In neurons, the rat MAGUIN proteins are localized in the cell body and in neurites where they are associated with the plasma-membrane via their PH-domains. Full-length rat MAGUIN-1 could be recovered from neuronal membrane fractions. Rat MAGUIN-1, but not rat MAGUIN-2, interacts via the PDZ-binding motif with the PDZ-domain of PSD-95/SAP90 and the synaptic scaffolding molecule (S-SCAM) (Hirao et al., *Journal of Biological Chemistry* 1998, 273: 21105-21110). S-SCAM has recently been identified as a multiple PDZ-domain containing protein, interacting with SAP90/PSD-95-associated protein (SAPAP), the NMDA receptor, and neuronal adhesion molecules. Additionally, interaction of the PH-domain of rat MAGUIN-1 with the kinase domain of Raf could be confirmed *in vitro* and *In vivo*, but their is no evidence for the activation of Raf or its recruitment to the plasma membrane by rat MAGUIN-1. To date, the precise function of rat MAGUIN-1 and rat MAGUIN-2 is still not clear. Some clues for the role of rat MAGUIN-1 in the cellular context come from particular protein domains and motifs, specific brain expression patterns, and structural homologies to other proteins like CNK (connector enhancer of kinase suppressor of ras (ksr)), as described for the fruit fly. CNK binds kinase suppressor for Ras and Raf kinase, functions in the Ras/MAP kinase pathway, and has been found to play a profound role in the regulation of eye development (Therrien et al., *Cell* 1998, 95: 343-353). On the basis of the similarities between *Drosophila* CNK (has SAM, PDZ and PH-domains) and rat MAGUIN-1, rat MAGUIN-1 is discussed as a rodent homolog to CNK.

Thus, it is likely that rat MAGUIN-1 assembles components of synaptic junctions and regulators of the MAP kinase pathway and links them to NMDA receptors and neuronal adhesion molecules. To date, no experiments have been described that show a relationship between a differential expression of human MAGUIN genes, neither on a transcriptional nor on a translational level, and the pathology of neurodegenerative disorders.

The disclosure in the present invention of the human MAGUIN-1 gene and the identification of a link of both human MAGUIN-1 and/or human MAGUIN-2 to neurodegenerative diseases, particularly Alzheimer's disease, offers new ways, inter alia, for the diagnosis and treatment of such diseases.

The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding

regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promotors or enhancers, introns, leader and trailer sequences). "Regulatory elements" as used in the present disclosure may include inducible and non-inducible promotors, enhancers, operators and other elements that drive and regulate gene expression.The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, acetylation or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene.

Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, ischemic stroke and age-related macular degeneration.

The invention features a novel isolated nucleic acid molecule encoding a protein molecule whose amino acid sequence comprises the sequence shown

in SEQ ID NO. 1. Hereinafter, the protein molecule of SEQ ID NO. 1 is denoted human MAGUIN-1, whereas the protein molecule of SEQ ID NO. 2, is denoted human Maguin-2. The amino acid sequences of the protein molecules of SEQ ID NO. 1 and SEQ ID NO. 2 comprise none, one, or several functionally important protein domains or modules. Subject to the protein modules of SEQ ID NO. 1 and SEQ ID NO. 2, human MAGUIN-1 and human Maguin-2 may function to organize and regulate synaptic signal transmission and nerve cell signal transduction. Both proteins may be implicated in information and storage processes and synaptic plasticity, and thus may play a role in neurodegeneration, in cell protection and regeneration processes. The present invention also features functional variants, derivatives and fragments of human MAGUIN-1, which might have a modification of the given primary structure of human MAGUIN-1, but whose essential biological function may remain unaffected.

The term "variant" as used herein refers to any polypeptide and protein, compared to the polypeptides and proteins used in the present invention, in which one or more amino acids are added and/or substituted and/or deleted and/or inserted at the N-terminus or C-terminus or within the native amino acid sequences of the native polypeptides and proteins of the present invention. Additionally, the term "variant" shall include any shorter version of the polypeptides and proteins herein. A variant shall also comprise a sequence that has at least about 85% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequences of SEQ ID NO. 1 and/or SEQ ID NO. 2, respectively. Derivatives, variants, and fragments may include, but are not limited to, a functional SAM, a functional PDZ and a functional PH domain or other functional modules within the polypeptide sequence of human MAGUIN-1 and human Maguin-2 proteins. Variants of a protein molecule shown in SEQ ID NO. 1 and/or SEQ ID NO. 2 may include, for example, proteins with conservative amino acid substitutions in highly conservative regions. For example, isoleucine, valine and leucine can each be substituted for one another. Aspartate and glutamate can be substituted for each other.

Glutamine and asparagine can be substituted for each other. Serine and threonine can be substituted for each other. Amino acid substitutions in less conservative regions include, for example, isoleucine, valine and leucine, which can each be substituted for one another. Aspartate and glutamate can be substituted for each other. Glutamine and asparagine can be subsituted for each other. Serine and threonine can be substituted for each other. Glycine and alanine can be substituted for each other. Alanine and valine can be substituted for each other. Methionine can be substituted for each of leucine, isoleucine or valine, and vice versa. Lysine and arginine can be substituted for each other. One of aspartate and glutamate can be substituted for one of arginine or lysine, and vice versa. Histidine can be substituted for arginine or lysine, and vice versa. Glutamine and glutamate can be substituted for each other. Asparagine and aspartate can be substituted for each other. Other examples of protein modifications include glycosylation and further post-translational modifications. Proteins and polypeptides of the present invention include variants, fragments and chemical derivatives of the protein comprising SEQ ID NO. 1 and/or SEQ ID NO. 2. They can include proteins and polypeptides which can be isolated from nature or which can be produced by recombinant and/or synthetic means. Native proteins or polypeptides refer to naturally-occurring truncated or secreted forms, naturally occurring variant forms (e.g. splice-variants) and naturally occurring allelic variants. As used herein, protein and polypeptide refers to a linear series of amino acid residues connected to one another by peptide bonds between the alpha-amino group and carboxy groups of adjacent amino acid residues. Other covalent bonds, such as amide and disulfide bonds, may also be present.

The invention also features nucleic acid molecules encoding functional variants of the protein molecule of SEQ ID NO. 1. Nucleic acid molecules can be DNA molecules, such as genomic DNA molecules or cDNA molecules, or RNA molecules, such as mRNA molecules. In particular, said nucleic acid molecules can be cDNA molecules comprising a nucleotide sequence of SEQ ID NO. 3.

The invention also features an isolated D N A molecule capable of hybridizing with the complement of the cD N A described in SEQ ID NO. 3 under stringent conditions. Stringent conditions means that hybridization will be carried out 5°C to 10°C below that temperature at which totally complementary nucleic acids will just-hybridize. Optimized stringency conditions for each sequence are established on parameters such as temperature, nucleic acid molecule consistency, salt conditions, and others well known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000). Examples for stringent conditions are (i) 0.2xSSC (standard saline citrate) and 0.1 % SDS at 60 °C and (ii) 50 % formamide, 4xSSC, 50 mM HEPES, pH 7.0, 10x Denhardt's solution, 100 µg/ml thermally denatured salmon sperm DNA at 42 °C. This shall not exclude even higher stringency conditions as mentioned, nor shall it exclude lower stringency conditions as mentioned.

In another aspect, the invention features a vector comprising a nucleic acid encoding a protein molecule shown in SEQ ID NO. 1. In preferred embodiments, a virus, a bacteriophage, or a plasmid comprises the described nucleic acid. In particular, a plasmid adapted for expression in a bacterial cell comprises said nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a fragment, or a variant thereof, and the regulatory elements necessary for expression of said molecule in a bacterial cell. In a further aspect, the invention features a plasmid adapted for expression in a yeast cell which comprises a nucleic acid molecule, encoding a protein molecule shown in SEQ ID NO. 1 or a variant or fragment thereof, and the regulatory elements necessary for expression of said molecule in a yeast cell. In another aspect, the invention features a plasmid adapted for expression in a mammalian cell which comprises a nucleic acid molecule, encoding a protein molecule shown in SEQ ID NO. 1 or a fragment or a variant thereof, and the regulatory elements necessary for expression of said molecule in a mammalian cell.

In a further aspect, the invention features a cell comprising a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a fragment or a variant thereof. The present invention also features cells comprising a DNA molecule capable of hybridizing with the complement of the cDNA described in SEQ ID NO. 3 under stringent conditions. In preferred embodiments, said cell is a bacterial cell, a yeast cell, a mammalian cell, or a cell of an insect. In particular, the invention features a bacterial cell comprising a plasmid adapted for expression in a bacterial cell, said plasmid comprising a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a fragment, or a variant thereof, and the regulatory elements necessary for expression of said molecule in a bacterial cell. The invention also features a yeast cell comprising a plasmid adapted for expression in a yeast cell, said plasmid comprises a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a fragment, or a variant thereof, and the regulatory elements necessary for expression of said molecule in a yeast cell. The invention further features a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, said plasmid comprising a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a variant, or a fragment thereof, and the regulatory elements necessary for expression of said molecule in a mammalian cell.

In one aspect the present invention features a protein molecule shown in SEQ ID NO. 1.

The invention further features an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the human MAGUIN-1 gene shown in SEQ ID NO. 1, or a fragment, a variant, or a derivative thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody.

Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized or single chain antibodies, as well as fragments thereof. Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods involving detecting of human MAGUIN-1 translation products.

The invention also features an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the human MAGUIN-2 gene shown in SEQ ID NO. 2, or a fragment, a variant, or a derivative thereof.

In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell. The invention is particularly suited to detect pathological structures in the brain of a subject. It is also especially suited to detect pathological cells of the muscular system, prostate, stomach, testis, ovary, adrenal glands, mammary glands, liver, spleen, lung, trachea or placenta. Preferably, the pathological state relates to a neurodegenerative disease, in particular to Alzheimer's disease. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the

cell, are carried out according to the method described in US patent 6150173.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or of (ii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or of (ii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value representing a known disease or health status. Thereby the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or of (ii) a translation product of a gene coding for human MAGUIN-1 and/or human

MAGUIN-2, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a preferred embodiment, said subjects suffer from Alzheimer's disease. Further examples of neurodegenerative diseases are Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, multiple system atrophy, cerebro-vascular dementia, and mild cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, ischemic stroke and age-related macular degeneration.

The present invention discloses the differential expression and regulation of the human MAGUIN-1 and/or human MAGUIN-2 gene in specific brain regions of Alzheimer's disease patients. Consequently, human MAGUIN-1 and/or human MAGUIN-2 and their corresponding translation products may have a causative role in the regional selective neuronal degeneration typically observed in Alzheimer's disease. Alternatively, human MAGUIN-1 and/or human MAGUIN-2 may confer a neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular Alzheimer's disease. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is particularly preferred that said sample to be analyzed and determined is selected from the group consisting of a brain tissue or other tissues, organs or body cells. The sample can also consist of cerebrospinal fluid or other body fluids including saliva, urine, blood, serum plasma, or nasal mucosa.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or of (ii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or of (iii) a fragment or derivative of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an increase or decrease in human MAGUIN-1 and/or human MAGUIN-2 mRNA and /or human MAGUIN-1 and/or human MAGUIN-2 protein in a sample cell or tissue from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of becoming diseased with a neurodegenerative disease, particularly Alzheimer's disease.

In preferred embodiments, measurement of the level of transcription products of a gene coding for a human MAGUIN-1 and/or human MAGUIN-2 is performed in a sample from a subject using a quantitative PCR-analysis with primer combinations to amplify said gene specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A Northern blot with probes specific for said gene can also be applied. It might also be preferred to measure transcription products by means of chip-based micro-array technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000).

Furthermore, the level of a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2 and/or fragment of said translation product, and/or level of activity of said translation product and/or fragment of said translation product, can be detected using an immunoassay, an activity assay and/or binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use

of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999). All these detection techniques may also be employed in the format of micro-arrays, protein-arrays, or protein-chip based technologies.

In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of human MAGUIN-1 and/or human MAGUIN-2, and/or of (ii) a translation product of human MAGUIN-1 and/or human MAGUIN-2, and/or of (iii) a fragment or derivative of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating Alzheimer's disease in a subject, or determining the propensity or predisposition of a subject to develop Alzheimer's disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2 (ii) reagents that selectively detect a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2; and

(b) instruction for diagnosing, or prognosticating Alzheimer's disease, or determining the propensity or predisposition of a subject to develop Alzheimer's disease by

- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, in a sample from said subject; and
- diagnosing or prognosticating Alzheimer's disease, or determining the propensity or predisposition of said subject to develop Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of Alzheimer's disease, or an increased propensity or predisposition of developing Alzheimer's disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing Alzheimer's disease. Consequently, the kit, according to the invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of Alzheimer's disease in a subject, as well as monitoring success or failure of therapeutic treatment for Alzheimer's disease of said subject.

The kit may also be applied for diagnosing or prognosticating a neurodegenerative disease related to Alzheimer's disease in a subject, or determining the propensity or predisposition of a subject to develop such a neurodegenerative disease, or monitoring a progression of such a neurodegenerative disease, or monitoring success or failure of a therapeutic treatment for such a neurodegenerative disease in a subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular Alzheimer's disease, in a subject comprising the administration to said subject in a therapeutically or

prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iii) a translation product of said gene, and/or (iv) a fragment or derivative of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence shown in SEQ ID NO.1 and/or SEQ ID NO.2, or a fragment thereof.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931; the contents of which are incorporated herein by reference) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane pertubation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar,

Trends Biotechnol 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against human MAGUIN-1 and/or human MAGUIN-2. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy.

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection, liposomal mediated transfection.

In preferred embodiments, said agent is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising

introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an animal model for a neurodegenerative disorder, e.g. a

transgenic mouse and/or a knock-out mouse with a neurodegenerative phenotype, in particular with an Alzheimer's-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2 and/or (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iv) a fragment or derivative of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iv) a fragment or derivative of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2 and/or (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iv) a fragment or derivative of (i) to (iii) for a

preparation of a medicament for treating or preventing a neurodegenerative disease, in particular Alzheimer's disease.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for human MAGUIN-1 and/or human MAGUIN-2, or a fragment, or a variant, or a derivative thereof. The generation of said recombinant, non-human animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar to a neurodegenerative disease, in particular Alzheimer's disease. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., 1994, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular Alzheimer's disease.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iv) a fragment or derivative of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iv) a fragment or derivative of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed said diseases and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the

activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant, non-human animal which expresses human MAGUIN-1 and/or human MAGUIN-2, or a fragment or a variant thereof, or a derivative thereof, under the control of a transcriptional regulatory element which is not the native human MAGUIN-1 and/or human MAGUIN-2 gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and human MAGUIN-1 and/or human MAGUIN-2, or a fragment or derivative thereof. Said screening assay comprises the steps of (i) adding a liquid suspension of said human MAGUIN-1 and/or human MAGUIN-2, or a fragment or derivative thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding fluorescently labelled ligand to said containers, and (iv) incubating said human MAGUIN-1 and/or human MAGUIN-2, or said fragment or derivative thereof, and said compound or plurality of compounds, and said fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said human MAGUIN-1 and/or human MAGUIN-2, or with said fragment or derivative thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said human MAGUIN-1 and/or human MAGUIN-2, or said fragment or derivative thereof. Instead of utilizing a

fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a gene product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, or a fragment or derivative thereof.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a gene product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2 by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to human MAGUIN-1 and/or human MAGUIN-2, or to a fragment, a variant, or derivative thereof. Said screening assay comprises (i) adding a liquid suspension of said human MAGUIN-1 and/or human MAGUIN-2, or a fragment, or a variant, or derivative thereof, to a plurality of containers, and (ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds to be screened for said binding to said plurality of containers, and (iii) incubating said human MAGUIN-1 and/or human MAGUIN-2, or said fragment, or variant, or derivative thereof, and said fluorescently labelled compound or fluorescently labelled compounds, and (iv) measuring the amounts of fluorescence associated with said human MAGUIN-1 and/or human MAGUIN-2, or with said fragment, or variant, or derivative thereof, and (v) determining the degree of binding by one or more of said compounds to said human MAGUIN-1 and/or human MAGUIN-2, or said fragment, or variant, or derivative thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other

type of detectable label might also be employed. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to a human MAGUIN-1 and/or human MAGUIN-2 gene product.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a gene product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2 by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

Other features and advantages of the invention will be apparent form the following description of figures and examples which are illustrative only, and not intended to limit the remainder of the disclosure in any way.

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in Alzheimer's disease. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in Alzheimer's disease (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in Alzheimer's disease. Brain tissues from the frontal cortex (F) and the temporal cortex (T) of Alzheimer's disease patients and healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the

image of a normal healthy brain was taken from a publication by Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figure 2 discloses the initial identification of the differential expression of the human MAGUIN-1 gene in a fluorescence differential display screen. The figure shows a clipping of a large preparative fluorescent differential display gel. PCR products from the frontal cortex (F) and the temporal cortex (T) of two healthy control subjects and six Alzheimer's diseased patients were loaded in duplicate onto a denaturing polyacrylamide gel (from left to right). PCR products were obtained by amplification of the individual cDNAs with the corresponding one-base-anchor oligonucleotide and the specific Cy3 labelled random primers. The arrow indicates the lane where significant differences in intensity of the signals for human MAGUIN-1 transcript derived from frontal cortex, compared to the signals for human MAGUIN-1 transcript derived from the temporal cortex of Alzheimer's patients exist. The differential expression reflects a down-regulation of human MAGUIN RNA expression in the temporal cortex in comparison to the frontal cortex of AD patients. Comparing the signals derived from frontal cortex and temporal cortex of healthy non-AD control subjects with each other, no distinction in signal intensity, i.e. no altered expression level can be detected.

Figure 3 and Figure 4 illustrate the verification of the differential expression of the human MAGUIN-1 and human MAGUIN-2 genes, respectively, by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and temporal cortex (T) of Alzheimer's disease patients (Figure 3b and Figure 4b) and of healthy, age-matched control individuals (Figure 3a and Figure 4a) was performed by the LightCycler™ rapid thermal cycling technique. The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for cyclophilin B, the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figure depicts the kinetics of

amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of human MAGUIN-1 and human MAGUIN-2 cDNAs from both the frontal and temporal cortices of a normal control individual during the exponential phase of the reaction overlap (Figure 3a and Figure 4a, arrowheads), whereas in Alzheimer's disease (Figure 3b and Figure 4b) there is a significant shift of the curve for the sample derived from temporal cortex, indicating a differential regulation of human MAGUIN-1 (Figure 3) and human MAGUIN-2 (Figure 4) mRNA expression in temporal cortex in comparison to frontal cortex.

Figure 5 discloses the protein sequence of human MAGUIN-1; SEQ ID NO. 1. The protein harbors several distinct functional domains which are located as follows: amino acid residues 8 to 75 form the ‚Sterile Alpha Motif' (SAM), amino acid residues 156 to 296 generate the PDZ domain, and the Pleckstrin-homology (PH) domain consist of amino acid residues 572 to 667.

Figure 6 shows an alignment of the amino acid sequence of SEQ ID NO.1, human MAGUIN-1, with rat MAGUIN-1 amino acid sequence (GenBank accession number aad04568). The full length human MAGUIN-1 protein consists of 1034 amino acids (residues given in the single-letter amino acid code).

Figure 7 discloses the protein sequence of human MAGUIN-2; SEQ ID NO. 2. The protein harbors several distinct functional domains which are located as follows: amino acid residues 8 to 75 form the ‚Sterile Alpha Motif' (SAM), amino acid residues 156 to 296 generate the PDZ domain, and the Pleckstrin-homology (PH) domain consist of amino acid residues 572 to 667.

Figure 8 shows an alignment of the amino acid sequence of SEQ ID NO. 2, human MAGUIN-2, with rat MAGUIN-2 amino acid sequence (GenBank accession number aad04567). The full length human MAGUIN-2 protein consists of 898 amino acids (residues given in the single-letter amino acid code).

Figure 9 represents the nucleotide sequence of SEQ ID NO. 3, the coding sequence of the human MAGUIN-1 gene, comprising 3105 nucleotides.

Figure 10 represents the nucleotide sequence of SEQ ID NO. 4, the coding sequence of the human MAGUIN-2 gene, comprising 2683 nucleotides.

Figure 11 shows SEQ ID NO. 5, the nucleotide sequence of the human MAGUIN-1 cDNA, comprising 5749 nucleotides.

Figure 12 shows SEQ ID NO. 6, the nucleotide sequence of the human MAGUIN-2 cDNA; comprising 4350 nucleotides.

Figure 13 depicts SEQ ID NO. 7, the nucleotide sequence of the 50 bp MAGUIN-1 cDNA fragment, identified and obtained by fluorescence differential display and subsequent cloning.

Figure 14 outlines the sequence alignment of SEQ ID NO. 7, the 50 bp human MAGUIN-1 cDNA fragment, with the 3'UTR nucleotide sequence of SEQ ID NO. 5 (nucleotide 5693 to 5742), the nucleotide sequence of human MAGUIN-1 cDNA.

Figure 15 charts the schematic alignment of SEQ ID NO. 7, the human MAGUIN-1 cDNA fragment, SEQ ID NO. 6, the human MAGUIN-2 cDNA sequence and the nucleotide sequence of SEQ ID NO. 5, the nucleotide sequence of human MAGUIN-1 cDNA, derived from the alignment of EST nucleotide sequences as found in the GenBank genetic sequence database. EST numbers are written on the left side, all sequences are 5' to 3' directed.

Table 1 lists the gene expression levels in the frontal cortex relative to the temporal cortex for the human MAGUIN-1 gene in seven Alzheimer's disease patients (1.42-4.17Δ fold) and five healthy, age-matched control individuals

(0.30-1.37Δ fold). The values shown are reciprocal values according to the formula described herein.

Table 2 lists the gene expression levels in the frontal cortex relative to the temporal cortex for the human MAGUIN-2 gene in seven Alzheimer's disease patients (1.77-11.73Δ fold) and five healthy, age-matched control individuals (0.30-1.42Δ fold). The values shown are reciprocal values according to the formula as described herein.

EXAMPLE I:

(i) Brain tissue dissection from patients with Alzheimer's disease:
Brain tissues from Alzheimer's disease patients and age-matched control subjects were collected within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Fig. 1) and stored at – 80 °C until RNA extractions were performed.

(ii) Isolation of total RNA:
Total RNA was extracted from post-mortem brain tissue by using the RNeasy™ kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality were determined with the DNA LabChip® system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were utilised to generate a melting curve with the LightCycler™ technology as described in the supplied protocol by the manufacturer (Roche).

(iii) cDNA synthesis and identification of differentially expressed genes by fluorescence differential display (FDD):

In order to identify changes in gene expression in different tissues we employed a modified and improved differential display (DD) screening method. The original DD screening method is known to those skilled in the art (Liang and Pardee, *Science* 1995, 267:1186-7). This technique compares two populations of RNA and provides clones of genes that are expressed in one population but not in the other. Several samples can be analyzed simultaneously and both up- and down-regulated genes can be identified in the same experiment. By adjusting and refining several steps in the DD method as well as modifying technical parameters, e.g. increasing redundancy, evaluating optimized reagents and conditions for reverse transcription of total RNA, optimizing polymerase chain reactions (PCR) and separation of the products thereof, a technique was developed which allows for highly reproducible and sensitive results. The applied and improved DD technique was described in detail by von der Kammer et al. (*Nucleic Acids Research* 1999, 27: 2211-2218). A set of 64 specifically designed random primers were developed (standard set) to achieve a statistically comprehensive analysis of all possible RNA species. Further, the method was modified to generate a preparative DD slab-gel technique, based on the use of fluorescently labelled primers. In the present invention, RNA populations from carefully selected post-mortem brain tissues (frontal and temporal cortex) of Alzheimer's disease patients and age-matched control subjects were compared.

As starting material for the DD analysis we used total RNA, extracted as described above (ii). Equal amounts of 0.05 µg RNA each were transcribed into cDNA in 20 µl reactions containing 0.5 mM each dNTP, 1 µl Sensiscript™ Reverse Transcriptase and 1x RT buffer (Qiagen), 10 U RNase inhibitor (Qiagen) and 1 µM of either one-base-anchor oligonucleotides $HT_{11}A$, $HT_{11}G$ or $HT_{11}C$ (Liang et al., *Nucleic Acids Research* 1994, 22: 5763-5764; Zhao et al., *Biotechniques* 1995, 18: 842-850). Reverse transcription was performed for 60 min at 37 °C with a final denaturation step at 93 °C for 5 min. 2 µl of the obtained cDNA each was subjected to a polymerase chain reaction (PCR) employing the corresponding one-base-

anchor oligonucleotide (1 µM) along with either one of the Cy3 labelled random DD primers (1 µM), 1x GeneAmp PCR buffer (Applied Biosystems), 1.5 mM $MgCl_2$ (Applied Biosystems), 2 µM dNTP-Mix (dATP, dGTP, dCTP, dTTP Amersham Pharmacia Biotech), 5 % DMSO (Sigma), 1 U AmpliTaq DNA Polymerase (Applied Biosystems) in a 20 µl final volume. PCR conditions were set as follows: one round at 94 °C for 30 sec for denaturing, cooling 1 °C/sec down to 40 °C, 40 °C for 4 min for low-stringency annealing of primer, heating 1 °C/sec up to 72 °C, 72 °C for 1 min for extension. This round was followed by 39 high-stringency cycles: 94 °C for 30 sec, cooling 1 °C/sec down to 60 °C, 60 °C for 2 min, heating 1 °C/sec up to 72 °C, 72 °C for 1 min. One final step at 72 °C for 5 min was added to the last cycle (PCR cycler: Multi Cycler PTC 200, MJ Research). 8 µl DNA loading buffer were added to the 20 µl PCR product preparation, denatured for 5 min and kept on ice until loading onto a gel. 3.5 µl each were separated on 0.4 mm thick, 6 %-polyacrylamide (Long Ranger)/ 7 M urea sequencing gels in a slab-gel system (Hitachi Genetic Systems) at 2000 V, 60W, 30 mA, for 1 h 40 min. Following completion of the electrophoresis, gels were scanned with a FMBIO II fluorescence-scanner (Hitachi Genetic Systems), using the appropriate FMBIO II Analysis 8.0 software. A full-scale picture was printed, differentially expressed bands marked, excised from the gel, transferred into 1.5 ml containers, overlayed with 200 µl sterile water and kept at –20°C until extraction.

Elution and reamplification of differential display products: The differential bands were extracted from the gel by boiling in 200 µl H_2O for 10 min, cooling down on ice and precipitation from the supernatant fluids by using ethanol (Merck) and glycogen/sodium acetate (Merck) at – 20 °C over night, and subsequent centrifugation at 13.000 rpm for 25 min at 4 °C. Pellets were washed twice in ice-cold ethanol (80%), resuspended in 10 mM Tris pH 8.3 (Merck) and dialysed against 10 % glycerol (Merck) for 1 h at room temperature on a 0.025 µm VSWP membrane (Millipore). The obtained preparations were used as templates for reamplification by 15 high-stringency cycles in 25-µl PCR mixtures containing the corresponding primer pairs as used for the differential display PCR (see above) under identical conditions,

with the exception of the initial round at 94 °C for 5 min, followed by 15 cycles of: 94 °C for 45 sec, 60 °C for 45 sec, ramp 1°C/sec to 70 °C for 45 sec, and one final step at 72 °C for 5 min.

Cloning and sequencing of differential display products: Re-amplified cDNAs were analyzed with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies) and were ligated into the pCR-Blunt II-TOPO vector and transformed into *E.coli* Top10F' cells (Zero Blunt TOPO PCR Cloning Kit, Invitrogen) according to the manufacturer's instructions. Cloned cDNA fragments were sequenced by commercially available sequencing facilities. The results of one such fluorescence differential display experiment for the human MAGUIN-1 gene are shown in Fig. 2.

(iv) Confirmation of differential expression by quantitative RT-PCR:

Positive corroboration of differential expression of the human MAGUIN-1 gene and human MAGUIN-2 gene was performed using the LightCycler technology (Roche). This technique features rapid thermal cyling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by using a kinetic, rather than an endpoint approach. The ratio of human MAGUIN-1 and human MAGUIN-2 cDNA from the temporal cortex and frontal cortex was determined (relative quantification).

First, a standard curve was generated to determine the efficiency of the PCR with specific primers for human MAGUIN-1 (5'-CAGCAAGCAGTTGACGGGA-3' and 5'-TGAATTTGACAAGACTCGTGGC-3') and human MAGUIN-2 (5'-GGGCCTCCC AAAGGGATAT-3' and 5'-CCCAATGTAGAAAGCTCGCATT-3'), respectively. PCR amplification (95 °C and 1 sec, 56 °C and 5 sec, and 72 °C and 5 sec) was performed in a volume of 20 µl containing Lightcycler-FastStart DNA Master SYBR Green I mix (contains FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and depending on the primers used, additional 3 mM $MgCl_2$. Melting curve analysis revealed a single peak at approximately 80.5°C

and 81°C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 120 bp for human MAGUIN-1 and 66 bp for human MAGUIN-2 was observed in the electropherogram of the sample.

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTT-TGCC-3' except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'-TCTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM $MgCl_2$ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (142 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTGTGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGGCTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number C_t for human MAGUIN-1 and human MAGUIN-2, respectively, and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from frontal cortex and temporal cortex were analyzed in parallel and normalized to cyclophilin B. The C_t values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10 \text{ ^ } ((C_t \text{ value - intercept}) / \text{slope}) \quad \text{[ng total brain cDNA]}$$

The values for frontal cortex and temporal cortex cDNAs of human MAGUIN-1 and human MAGUIN-2 cDNAs were normalized to cyclophilin B, and the ratio was calculated using the following formula:

$$\text{Ratio} = \frac{\text{human MAGUIN-1 temporal [ng] / cyclophilin B temporal [ng]}}{\text{human MAGUIN-1 frontal [ng] / cyclophilin B frontal [ng]}}$$

$$\text{Ratio} = \frac{\text{human MAGUIN-2 temporal [ng] / cyclophilin B temporal [ng]}}{\text{human MAGUIN-2 frontal [ng] / cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in step 2 and step 3, and the ratio from one gene to another gene remained constant in different runs, it was possible to normalize the values for human MAGUIN-1 and human MAGUIN-2 to the mean average value of the set of reference standard genes instead of

normalizing to one single gene alone. The calculation was performed by dividing the ratio shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes. The results of one such quantitative RT-PCR analysis for the human MAGUIN-1 gene are shown in Figure 3 and for the human MAGUIN-2 gene in Figure 4.

(v) Sequence Analysis

Searching the EST database of the GenBank database for sequence similarities to the identified differentially expressed human cDNA fragment, SEQ ID NO. 7, as stated in the present invention, it was found that SEQ ID NO. 7 was identical to portions of the human EST sequences ai817268 and bf115709, (shown in Figure 15). Those human ESTs showed high homology to rat (*Rattus norvegicus)* MAGUIN-1. Aligning human ESTs in addition to the identified expressed SEQ ID NO. 7, a complete EST cluster representing the human MAGUIN-1 cDNA, SEQ ID NO. 5, was constructed. The amino acid sequence of a large open reading frame with the potential to encode a protein of 1034 amino acid residues was deduced (SEQ ID NO. 1). The human MAGUIN-1 protein, as denoted herein, is highly homologous to the rat MAGUIN-1 protein. In addition, it encodes a protein (SEQ ID NO. 1) harboring a number of structurally and functionally important domains. One SAM, one PDZ, and one PH domain are located from amino acid residues 8 to 75 (SAM), amino acid residues 156 to 296 (PDZ), and amino acid residues 572 to 667 (PH) (refer to Figure 4).

CLAIMS

1. An isolated nucleic acid encoding a protein molecule shown in SEQ ID NO. 1.

2. An isolated nucleic acid molecule of claim 1, wherein the nucleic acid molecule is a D N A molecule.

3. An isolated nucleic acid molecule of claim 2, wherein the nucleic acid molecule is a cD N A molecule, in particular a cD N A molecule comprising a nucleotide sequence shown in SEQ ID NO. 3.

4. An isolated D N A molecule capable of hybridizing with the complement of the cD N A described in SEQ ID NO. 3 under stringent condition.

5. A vector comprising a nucleic acid molecule according to one of claims 1 to 4.

6. A vector according to claim 5 wherein said vector is a plasmid, a virus, or a bacteriophage.

7. A plasmid according to claim 6 wherein said plasmid is adapted for expression in a yeast cell and further comprises the regulatory elements necessary for expression of said nucleic acid molecule.

8. A plasmid according to claim 6 wherein said plasmid is adapted for expression in a bacterial cell and further comprises the regulatory elements necessary for expression of said nucleic acid molecule.

9. A plasmid according to claim 6 wherein said plasmid is adapted for expression in a mammalian cell and further comprises the regulatory elements necessary for expression of said nucleic acid molecule.

10. A cell transformed with a nucleic acid molecule according to one of claims 1 to 4, wherein said cell is in particular a bacterial cell, a yeast cell, a mammalian cell, or an insect cell.

11. A protein molecule shown in SEQ ID NO. 1.

12. An antibody specifically immunoreactive with an immunogen, wherein said immunogen is a protein molecule shown in SEQ ID NO. 1, or a fragment thereof.

13. An antibody specifically immunoreactive with an immunogen, wherein said immunogen is a protein molecule shown in SEQ ID NO. 2, or a fragment thereof.

14. Use of an antibody of claim 12 or 13, for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell representing a known health status indicates a pathological state of said cell, and wherein said pathological state relates to a neurodegenerative disease, preferably Alzheimer's disease.

15. A method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or

(ii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or

(iii) a fragment or derivative of said transcription or translation product,

in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

16. A method of monitoring the progression of a neurodegenerative disease in a subject, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or

(ii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or

(iii) a fragment or derivative of said transcription or translation product,

in a sample from said subject and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby monitoring the progression of said neurodegenerative disease in said subject.

17. A method of evaluating a treatment for a neurodegenerative disease, comprising:

determining a level and/or an activity of

(i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or

(ii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or

(iii) a fragment or derivative of said transcription or translation product,

in a sample from a subject being treated for said disease and comparing said level and/or said activity to a reference value representing a known disease or health status, thereby evaluating said treatment for said neurodegenerative disease.

18. The method according to any of claims 15 to 17, wherein said neurodegenerative disease is Alzheimer's disease.

19. The method according to any of claims 15 to 18, wherein said sample is a cell, or a tissue, or an organ, or a body fluid, in particular cerebrospinal fluid or blood.

20. The method according to any of claims 15 to 19, wherein said reference value is that of a level and/or an activity of
(i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or
(ii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or
(iii) a fragment or derivative of said transcription or translation product, in a sample from a subject not suffering from said neurodegenerative disease.

21. The method according to any of claims 15 to 20, wherein an increase or decrease in human MAGUIN-1 RNA and/or human MAGUIN-2 RNA and/or human MAGUIN-1 protein and/or human MAGUIN-2 protein in a cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of Alzheimer's disease in said subject.

22. The method according to any of claims 15 to 21, further comprising comparing a level and/or an activity of
(i) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or

(ii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or

(iii) a fragment or derivative of said transcription or translation product

in a series of samples taken from said subject over a period of time.

23. The method according to claim 22, wherein said subject receives a treatment prior to one or more of said sample gatherings.

24. The method according to claim 23 wherein said level and/or activity is determined before and after said treatment of said subject.

25. A kit for diagnosing or prognosticating Alzheimer's disease, in a subject, or determining the propensity or predisposition of a subject to develop Alzheimer's disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of

(i) reagents that selectively detect a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and (ii) reagents that selectively detect a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2 and

(b) instruction for diagnosing, or prognosticating Alzheimer's disease, or determining the propensity or predisposition of a subject to develop Alzheimer's disease by

- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, in a sample from said subject; and
- diagnosing or prognosticating Alzheimer's disease, or determining the propensity or predisposition of said subject to develop Alzheimer's disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status;

or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status indicates a diagnosis or prognosis of Alzheimer's disease, or an increased propensity or predisposition of developing Alzheimer's disease.

26. A method of treating or preventing a neurodegenerative disease, in particular Alzheimer's disease, in a subject comprising administering to said subject in a therapeutically or prophylactically effective amount an agent or agents which directly or indirectly affect an activity and/or a level of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iv) a fragment or derivative of (i) to (iii).

27. A modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iv) a fragment or derivative of (i) to (iii).

28. Use of a modulator of an activity and/or of a level of at least one substance which is selected from the group consisting of (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or (iv) a fragment or derivative of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular Alzheimer's disease.

29. A recombinant, non-human animal comprising a non-native gene sequence coding for human MAGUIN-1 and/or human MAGUIN-2 or a fragment thereof, or a derivative thereof, said animal being obtainable by:
    (i) providing a gene targeting construct comprising said gene sequence and a selectable marker sequence, and
    (ii) introducing said targeting construct into a stem cell of a non-human animal, and
    (iii) introducing said non-human animal stem cell into a non-human embryo, and
    (iv) transplanting said embryo into a pseudopregnant non-human animal, and
    (v) allowing said embryo to develop to term, and
    (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and
    (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said disruption results in said non-human animal exhibiting a predisposition to developing a neurodegenerative disease or related diseases or disorders.

30. An assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases or disorders of one or more substances selected from the group consisting of
    (i) a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or
    (ii) a transcription product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or
    (iii) a translation product of a gene coding for human MAGUIN-1 and/or human MAGUIN-2, and/or
    (iv) a fragment or derivative of (i) to (iii), said method comprising:
    (a) contacting a cell with a test compound;

(b) measuring the activity and/or level of one or more substances recited in (i) to (iv);

(c) measuring the activity and/or level of one or more substances recited in (i) to (iv) in a control cell not contacted with said test compound; and

comparing the levels and/or activities of the substance in the cells of step (b) and (c), wherein an alteration in the activity and/or level of substances in the contacted cells indicates that the test compound is a modulator of said diseases or disorders.

SUMMARY

The present invention discloses a novel nucleic acid molecule encoding human MAGUIN-1 protein. Further, the invention discloses the differential expression of the human MAGUIN-1 and/or human MAGUIN-2 gene in specific brain regions of Alzheimer's disease patients. Based on this finding, this invention provides a method for diagnosing or prognosticating Alzheimer's disease in a subject, or for determining whether a subject is at increased risk of developing Alzheimer's disease. Furthermore, this invention provides therapeutic and prophylactic methods for treating or preventing Alzheimer's disease and related neurodegenerative disorders using a human MAGUIN gene. A method of screening for modulating agents of neurodegenerative diseases is also disclosed.

Figure 1: Identification of Genes Involved in Alzheimer's Disease Pathology

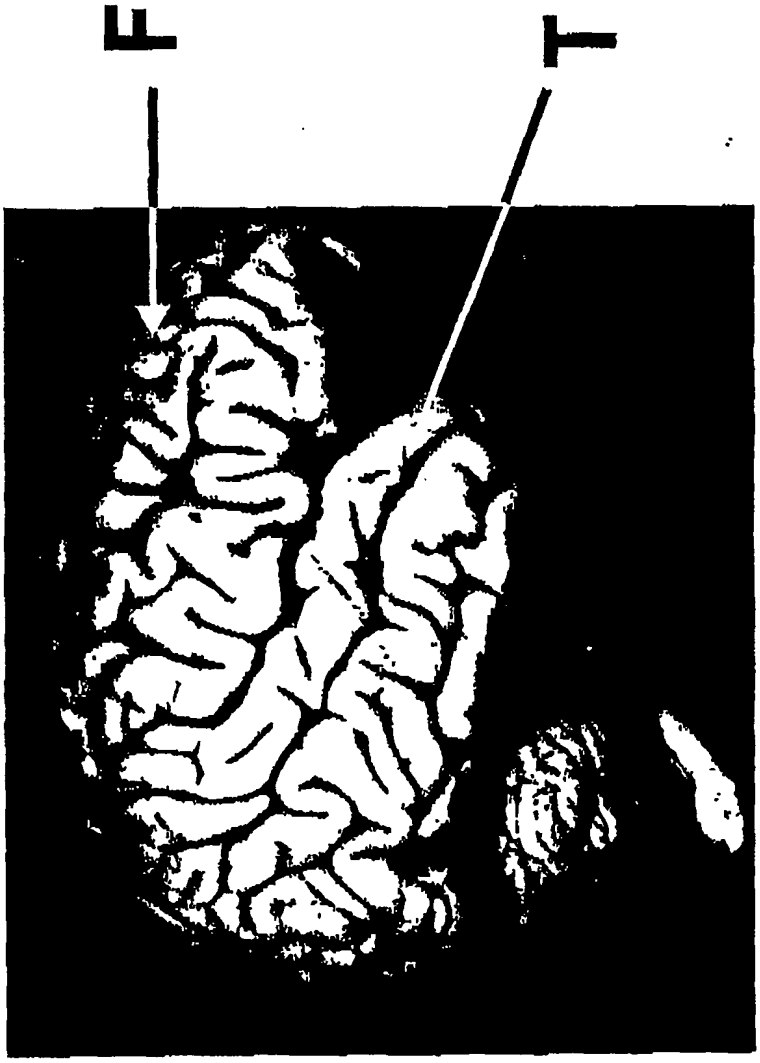

Figure 2: Identification of differentially expressed genes in a fluorescence differential display screen

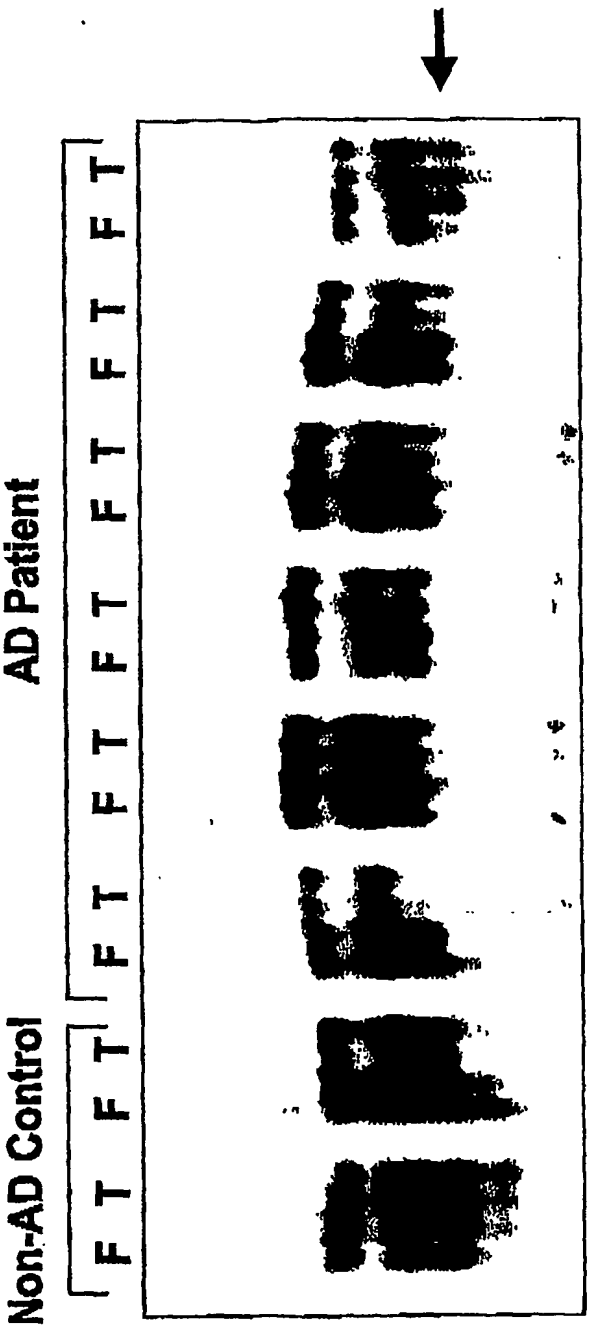

Figure 3: Verification of differential expression of human MAGUIN-1 by quantitative RT-PCR

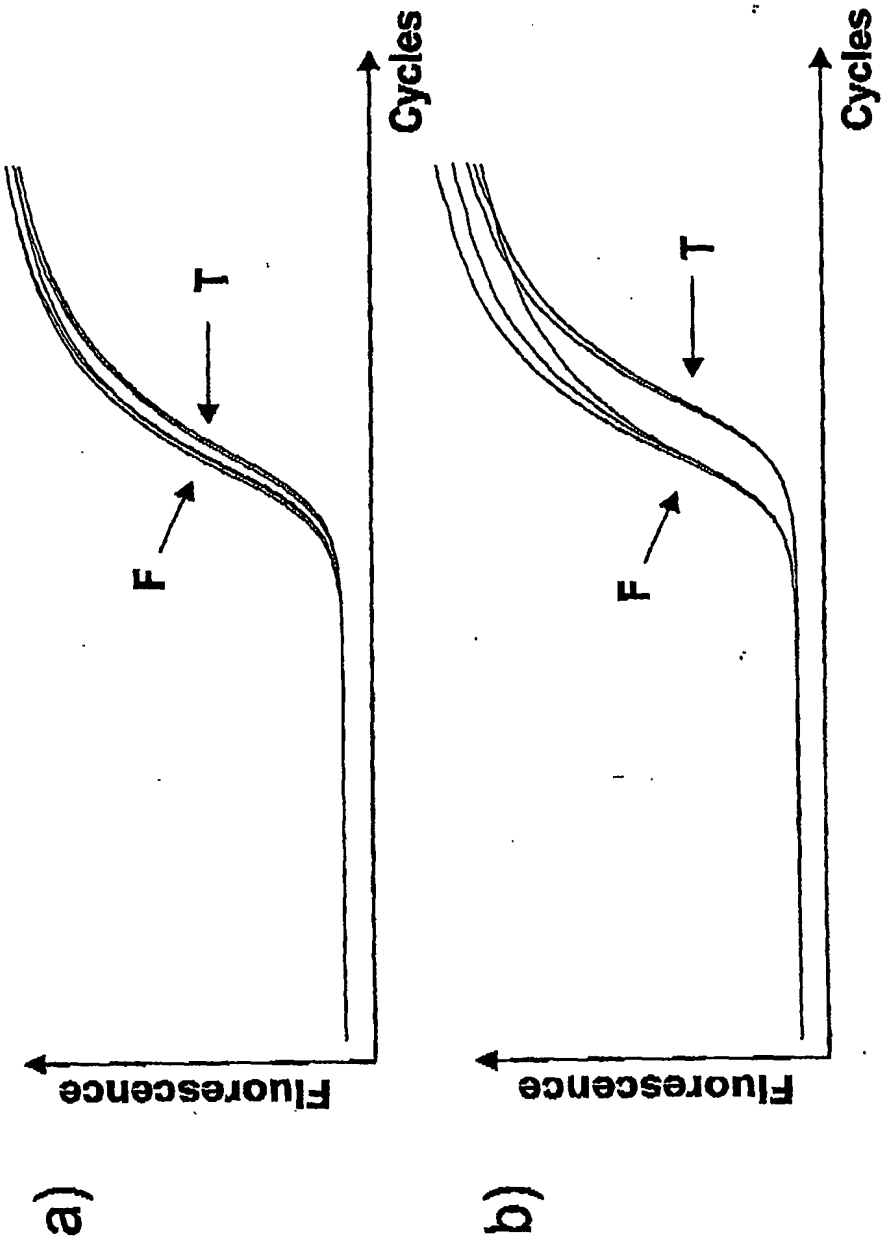

Figure 4: Verification of differential expression of human MAGUIN-2 by quantitative RT-PCR

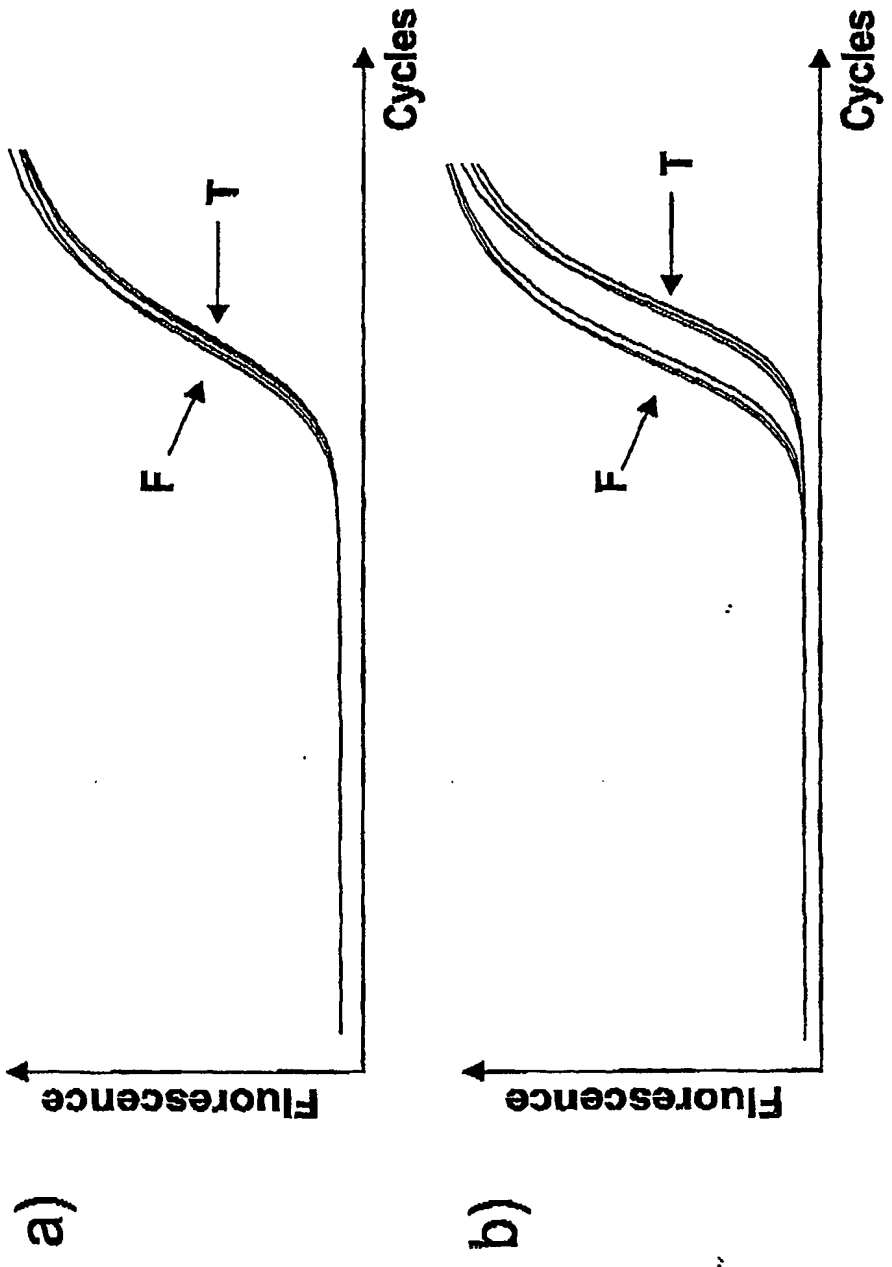

Figure 5: SEQ ID NO. 1: amino acid sequence of human MAGUIN-1 protein

Length: 1034 aa

1 MALIMEPVSK WSPSQVVDWM KGLDDCLQQY IKNFEREKIS GDQLLRITHQ

51 ELEDLGVSRI GHQELILEAV DLLCALNYGL ETENLKTLSH KLNASAKNLQ

101 NFITGRRRSG HYDGRTSRKL PNDFLTSVVD LIGAAKSLLA WLDRSPFAAV

151 TDYSVTRNNV IQLCLELTTI VQQDCTVYET ENKILHVCKT LSGVCDHIIS

201 LSSDPLVSQS AHLEVIQLAN IKPSEGLGMY IKSTYDGLHV ITGTTENSPA

251 DRCKKIHAGD EVIQVNHQTV VGWQLKNLVN ALREDPSGVI LTLKKRPQSM

301 LTSAPALLKN MRWKPLALQP LIPRSPTSSV ATPSSTISTP TKRDSSALQD

351 LYIPPPPAEP YIPRDEKGNL PCEDLRGHMV GKPVHKGSES PNSFLDQEYR

401 KRFNIVEEDT VLYCYEYEKG RSSSQGRRES TPTYGKLRPI SMPVEYNWVG

451 DYEDPNKMKR DSRRENSLLR YMSNEKIAQE EYMFQRNSKK DTGKKSKKKG

501 DKSNSPTHYS LLPSLQMDAL RQDIMGTPVP ETTLYHTFQQ SSLQHKSKKK

551 NKGPIAGKSK RRISCKDLGR GDCEGWLWKK KDAKSYFSQK WKKYWFVLKD

601 ASLYWYINEE DEKAEGFISL PEFKIDRASE CRKKYAFKAC HPKIKSFYFA

651 AEHLDDMNRW LNRINMLTAG YAERERIKQE QDYWSESDKE EADTPSTPKQ

701 DSPPPPYDTY PRPPSMSCAS PYVEAKHSRL SSTETSQSQS SHEEFRQEVT

751 GSSAVSPIRK TASQRRSWQD LIETPLTSSG LHYLQTLPLE DSVFSDSAAI

801 SPEHRRQSTL PTQKCHLQDH YGPYPLAESE RMQVLNGNGG KPRSFTLPRD

851 SGFNHCCLNA PVSACDPQDD VQPPEVEEEE EEEEEEGEAA GENIGEKSES

901 REEKLGDSLQ DLYRALEQAS LSPLGEHRIS TKMEYKLSFI KRCNDPVMNE

951 KLHRLRILKS TLKAREGEVA IIDKVLDNPD LTSKEFQQWK QMYLDLFLDI

1001 CQNTTSNDPL SISSEVDVIT SSLAHTHSYI ETHV*

Figure 6: Alignment of SEQ ID NO. 1, human MAGUIN-1, with rat MAGUIN-1

Length: 1034 aa

```
  1 MALIMEPVSKWSPSQVVDWMKGLDDCLQQYIKNFEREKISGDQLLRITHQ 50
    ||||||||||||||||||||||||||||||||||||||||||||||||||
  1 MALIMEPVSKWSPSQVVDWMKGLDDCLQQYIKNFEREKISGDQLLRITHQ 50

 51 ELEDLGVSRIGHQELILEAVDLLCALNYGLETENLKTLSHKLNASAKNLQ 100
    ||||||||||||||||||||||||||||||||||||||||||||||||||
 51 ELEDLGVSRIGHQELILEAVDLLCALNYGLETENLKTLSHKLNASAKNLQ 100

101 NFITGRRRSGHYDGRTSRKLPNDFLTSVVDLIGAAKSLLAWLDRSPFAAV 150
    ||||||||||||||||||||||||||||||||||||||||||||||||||
101 NFITGRRRSGHYDGRTSRKLPNDFLTSVVDLIGAAKSLLAWLDRSPFAAV 150

151 TDYSVTRNNVIQLCLELTTIVQQDCTVYETENKILHVCKTLSGVCDHIIS 200
    ||||||||||||||||||||||||||||||||||||||||||||||||||
151 TDYSVTRNNVIQLCLELTTIVQQDCTVYETENKILHVCKTLSGVCDHIIS 200

201 LSSDPLVSQSAHLEVIQLANIKPSEGLGMYIKSTYDGLHVITGTTENSPA 250
    ||||||||||||||||||||||||||||||||||||||||||||||||||
201 LSSDPLVSQSAHLEVIQLANIKPSEGLGMYIKSTYDGLHVITGTTENSPA 250

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251 DRCKKIHAGDEVIQVNHQTVVGWQLKNLVNALREDPSGVILTLKKRPQSM 300

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    ||||||||||||||||||||||||||||||||||||||||||||||||||
301 LTSAPALLKNMRWKPLALQPLIPRSPTSSVATPSSTISTPTKRDSSALQD 350

351 LYIPPPPAEPYIPRDEKGNLPCEDLRGHMVGKPVHKGSESPNSFLDQEYR 400
    ||||||||||||||||||||||||||||||||||||||||||||||||||
351 LYIPPPPAEPYIPRDEKGNLPCEDLRGHMVGKPVHKGSESPNSFLDQEYR 400

401 KRFNIVEEDTVLYCYEYEKGRSSSQGRRESTPTYGKLRPISMPVEYNWVG 450
    ||||||||||||||||||||||||||||||||||||||||||||||||||
401 KRFNIVEEDTVLYCYEYEKGRSSSQGRRESTPTYGKLRPISMPVEYNWVG 450

451 DYEDPNKMKRDSRRENSLLRYMSNEKIAQEEYMFQRNSKKDTGKKSKKKG 500
    ||||||||||||||||||||||||||||||||||||||||||||||||||
451 DYEDPNKMKRDSRRENSLLRYMSNEKIAQEEYMFQRNSKKDTGKKSKKKG 500
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```
501 DKSNSPTHYSLLPSLQMDALRQDIMGTPVPETTLYHTFQQSSLQHKSKKK 550
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501 DKSTSPTHYSLLPSLQMDALRQDIMGTPVPETTLYHTFQQSSLQHKSKKK 550

551 NKGPIAGKSKRRISCKDLGRGDCEGWLWKKKDAKSYFSQKWKKYWFVLKD 600
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551 NKGAIAGKSKRRISCKDLGRGDCEGWLWKKKDAKSYFSQKWKKYWFVLKD 600

601 ASLYWYINEEDEKAEGFISLPEFKIDRASECRKKYAFKACHPKIKSFYFA 650
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601 ASLYWYINEEDEKAEGFISLPEFKIDRASECRKKYAFKACHPKIKSFYFA 650

651 AEHLDDMNRWLNRINMLTAGYAERERIKQEQDYWSESDKEEADTPSTPKQ 700
    ||||||||||||||||||||||||||||||||||||||||||||||||||
651 AEHLDDMNRWLNRINMLTAGYAERERIKQEQDYWSESDKEEADTPSTPKQ 700

701 DSPPPPYDTYPRPPSMSCASPYVEAKHSRLSSTETSQSQSSHEEFRQEVT 750
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701 DSPPPPYDTYPRPPSMSCASPYVEAKHSRLSSTETSQSQSSHEEFRQEVT 750

751 GSSAVSPIRKTASQRRSWQDLIETPLTSSGLHYLQTLPLEDSVFSDSAAI 800
    ||||||||||||||||||||||||||||||||||||||||||||||||||
751 GSSAVSPIRKTASQRRSWQDLIETPLTSSGLHYLQTLPLEDSVFSDSAAI 800

801 SPEHRRQSTLPTQKCHLQDHYGPYPLAESERMQVLNGNGGKPRSFTLPRD 850
    ||||||||||||||||||||||||||||||||||||||||||||||||||
801 SPEHRRQSTLPTQKCHLQDHYGPYPLAESERMQVLNGNGGKPRSFTLPRD 850

851 SGFNHCCLNAPVSACDPQDDVQPPEVEEEEEEEEEEGEAAGENIGEKSES 900
    ||||||||||||||||||:|||||||||||||||  ||||||||||:|:
851 SGFNHCCLNAPVSACDPQDDIQPPEVEEEEEEEEE..EAAGENIGEKNEN 898

901 REEKLGDSLQDLYRALEQASLSPLGEHRISTKMEYKLSFIKRCNDPVMNE 950
    ||||||||||||||||:|||||||||||||||:||||||||||||||||
899 REEKLGDSLQDLYRALEEASLSPLGEHRISTKIEYKLSFIKRCNDPVMNE 948

951 KLHRLRILKSTLKAREGEVAIIDKVLDNPDLTSKEFQQWKQMYLDLFLDI 1000
    ||||||||||||||||||||||||||||||||||||||||||||||||||
949 KLHRLRILKSTLKAREGEVAIIDKVLDNPDLTSKEFQQWKQMYLDLFLDI 998

1001 CQNTTSNDPLSISSEVDVITSSLAHTHSYIETHV 1034
     ||||||||||||||||||||||||:||||||||||
 999 CQNTTSNDPLSISSEVDVITSSLTHTHSYIETHV 1032
```

Figure 7 : SEQ ID NO. 2: amino acid sequence of human MAGUIN-2 protein

Length: 898 aa

1 MALIMEPVSK WSPSQVVDWM KGLDDCLQQY IKNFEREKIS GDQLLRITHQ

51 ELEDLGVSRI GHQELILEAV DLLCALNYGL ETENLKTLSH KLNASAKNLQ

101 NFITGRRRSG HYDGRTSRKL PNDFLTSVVD LIGAAKSLLA WLDRSPFAAV

151 TDYSVTRNNV IQLCLELTTI VQQDCTVYET ENKILHVCKT LSGVCDHIIS

201 LSSDPLVSQS AHLEVIQLAN IKPSEGLGMY IKSTYDGLHV ITGTTENSPA

251 DRCKKIHAGD EVIQVNHQTV VGWQLKNLVN ALREDPSGVI LTLKKRPQSM

301 LTSAPALLKN MRWKPLALQP LIPRSPTSSV ATPSSTISTP TKRDSSALQD

351 LYIPPPPAEP YIPRDEKGNL PCEDLRGHMV GKPVHKGSES PNSFLDQEYR

401 KRFNIVEEDT VLYCYEYEKG RSSSQGRRES TPTYGKLRPI SMPVEYNWVG

451 DYEDPNKMKR DSRRENSLLR YMSNEKIAQE EYMFQRNSKK DTGKKSKKKG

501 DKSNSPTHYS LLPSLQMDAL RQDIMGTPVP ETTLYHTFQQ SSLQHKSKKK

551 NKGPIAGKSK RRISCKDLGR GDCEGWLWKK KDAKSYFSQK WKKYWFVLKD

601 ASLYWYINEE DEKAEGFISL PEFKIDRASE CRKKYAFKAC HPKIKSFYFA

651 AEHLDDMNRW LNRINMLTAG YAERERIKQE QDYWSESDKE EADTPSTPKQ

701 DSPPPPYDTY PRPPSMSCAS PYVEAKHSRL SSTETSQSQS SHEEFRQEVT

751 GSSAVSPIRK TASQRRSWQD LIETPLTSSG LHYLQTLPLE DSVFSDSAAI

801 SPEHRRQSTL PTQKCHLQDH YGPYPLAESE RMQVLNGNGG KPRSFTLPRD

851 SGFNHCCLNA PVSACDPQDD VQPPEVEEEE EEEEEEGEAA GENIGEKS*

Figure 8: Alignment of SEQ ID NO. 2, human MAGUIN-2, with rat MAGUIN-2

Length: 898 aa

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  1 MALIMEPVSKWSPSQVVDWMKGLDDCLQQYIKNFEREKISGDQLLRITHQ  50
    ||||||||||||||||||||||||||||||||||||||||||||||||||
  1 MALIMEPVSKWSPSQVVDWMKGLDDCLQQYIKNFEREKISGDQLLRITHQ  50

 51 ELEDLGVSRIGHQELILEAVDLLCALNYGLETENLKTLSHKLNASAKNLQ 100
    ||||||||||||||||||||||||||||||||||||||||||||||||||
 51 ELEDLGVSRIGHQELILEAVDLLCALNYGLETENLKTLSHKLNASAKNLQ 100

101 NFITGRRRSGHYDGRTSRKLPNDFLTSVVDLIGAAKSLLAWLDRSPFAAV 150
    ||||||||||||||||||||||||||||||||||||||||||||||||||
101 NFITGRRRSGHYDGRTSRKLPNDFLTSVVDLIGAAKSLLAWLDRSPFAAV 150

151 TDYSVTRNNVIQLCLELTTIVQQDCTVYETENKILHVCKTLSGVCDHIIS 200
    ||||||||||||||||||||||||||||||||||||||||||||||||||
151 TDYSVTRNNVIQLCLELTTIVQQDCTVYETENKILHVCKTLSGVCDHIIS 200

201 LSSDPLVSQSAHLEVIQLANIKPSEGLGMYIKSTYDGLHVITGTTENSPA 250
    ||||||||||||||||||||||||||||||||||||||||||||||||||
201 LSSDPLVSQSAHLEVIQLANIKPSEGLGMYIKSTYDGLHVITGTTENSPA 250

251 DRCKKIHAGDEVIQVNHQTVVGWQLKNLVNALREDPSGVILTLKKRPQSM 300
    ||||||||||||||||||||||||||||||||||||||||||||||||||
251 DRCKKIHAGDEVIQVNHQTVVGWQLKNLVNALREDPSGVILTLKKRPQSM 300

301 LTSAPALLKNMRWKPLALQPLIPRSPTSSVATPSSTISTPTKRDSSALQD 350
    ||||||||||||||||||||||||||||||||||||||||||||||||||
301 LTSAPALLKNMRWKPLALQPLIPRSPTSSVATPSSTISTPTKRDSSALQD 350

351 LYIPPPPAEPYIPRDEKGNLPCEDLRGHMVGKPVHKGSESPNSFLDQEYR 400
    ||||||||||||||||||||||||||||||||||||||||||||||||||
351 LYIPPPPAEPYIPRDEKGNLPCEDLRGHMVGKPVHKGSESPNSFLDQEYR 400

401 KRFNIVEEDTVLYCYEYEKGRSSSQGRRESTPTYGKLRPISMPVEYNWVG 450
    ||||||||||||||||||||||||||||||||||||||||||||||||||
401 KRFNIVEEDTVLYCYEYEKGRSSSQGRRESTPTYGKLRPISMPVEYNWVG 450

451 DYEDPNKMKRDSRRENSLLRYMSNEKIAQEEYMFQRNSKKDTGKKSKKKG 500
    ||||||||||||||||||||||||||||||||||||||||||||||||||
451 DYEDPNKMKRDSRRENSLLRYMSNEKIAQEEYMFQRNSKKDTGKKSKKKG 500
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501 DKSNSPTHYSLLPSLQMDALRQDIMGTPVPETTLYHTFQQSSLQHKSKKK 550
    |||:||||||||||||||||||||||||||||||||||||||||||||||||
501 DKSTSPTHYSLLPSLQMDALRQDIMGTPVPETTLYHTFQQSSLQHKSKKK 550

551 NKGPIAGKSKRRISCKDLGRGDCEGWLWKKKDAKSYFSQKWKKYWFVLKD 600
    |||:||||||||||||||||||||||||||||||||||||||||||||||||
551 NKGAIAGKSKRRISCKDLGRGDCEGWLWKKKDAKSYFSQKWKKYWFVLKD 600

601 ASLYWYINEEDEKAEGFISLPEFKIDRASECRKKYAFKACHPKIKSFYFA 650
    ||||||||||||||||||||||||||||||||||||||||||||||||||
601 ASLYWYINEEDEKAEGFISLPEFKIDRASECRKKYAFKACHPKIKSFYFA 650

651 AEHLDDMNRWLNRINMLTAGYAERERIKQEQDYWSESDKEEADTPSTPKQ 700
    ||||||||||||||||||||||||||||||||||||||||||||||||||
651 AEHLDDMNRWLNRINMLTAGYAERERIKQEQDYWSESDKEEADTPSTPKQ 700

701 DSPPPPYDTYPRPPSMSCASPYVEAKHSRLSSTETSQSQSSHEEFRQEVT 750
    ||||||||||||||||||||||||||||||||||||||||||||||||||
701 DSPPPPYDTYPRPPSMSCASPYVEAKHSRLSSTETSQSQSSHEEFRQEVT 750

751 GSSAVSPIRKTASQRRSWQDLIETPLTSSGLHYLQTLPLEDSVFSDSAAI 800
    ||||||||||||||||||||||||||||||||||||||||||||||||||
751 GSSAVSPIRKTASQRRSWQDLIETPLTSSGLHYLQTLPLEDSVFSDSAAI 800

801 SPEHRRQSTLPTQKCHLQDHYGPYPLAESERMQVLNGNGGKPRSFTLPRD 850
    ||||||||||||||||||||||||||||||||||||||||||||||||||
801 SPEHRRQSTLPTQKCHLQDHYGPYPLAESERMQVLNGNGGKPRSFTLPRD 850

851 SGFNHCCLNAPVSACDPQDDVQPPEVEEEEEEEEEGEAAGENIGEKS  898
    ||||||||||||||||||:|||||||||||||  ||||||||||
851 SGFNHCCLNAPVSACDPQDDIQPPEVEEEEEEEE..EAAGENIGEKS  896
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Figure 9: SEQ ID NO. 3: nucleotide sequence of human MAGUIN-1 coding sequence

Length: 3105 bp

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   1 ATGGCTCTGA TAATGGAACC GGTGAGCAAA TGGTCTCCGA GTCAAGTAGT GGACTGGATG
  61 AAAGGTCTTG ATGACTGTTT GCAGCAGTAT ATTAAGAACT TTGAGAGGGA GAAGATCAGT
 121 GGGGACCAGC TGCTGCGCAT TACACATCAG GAGCTAGAAG ATCTGGGGGT CAGCCGCATT
 181 GGCCATCAGG AACTGATCTT GGAAGCAGTT GACCTTCTGT GTGCATTGAA TTATGGCTTG
 241 GAAACAGAAA ATCTAAAAAC CCTTTCTCAC AAGTTGAATG CATCTGCCAA AAATCTGCAG
 301 AATTTTATAA CAGGAAGGAG AAGGAGTGGC CATTATGATG GGAGGACCAG CCGAAAATTG
 361 CCAAACGACT TTCTGACCTC AGTTGTGGAT CTGATTGGAG CAGCCAAGAG TCTGCTTGCC
 421 TGGTTGGACA GGTCACCATT TGCTGCTGTG ACAGACTATT CAGTTACAAG AAATAATGTC
 481 ATACAACTCT GCCTGGAGTT AACAACAATT GTGCAACAGG ATTGTACTGT ATATGAAACA
 541 GAGAATAAAA TTCTTCACGT GTGTAAAACT CTTTCTGGAG TCTGTGACCA CATCATATCC
 601 CTGTCGTCAG ATCCTCTGGT TTCACAGTCT GCTCACCTGG AAGTGATTCA ACTGGCAAAC
 661 ATTAAACCAA GCGAAGGGCT GGGTATGTAT ATTAAATCTA CATATGATGG CCTCCATGTA
 721 ATTACTGGAA CCACAGAAAA TTCACCTGCA GATCGGTGCA AGAAAATCCA TGCTGGCGAT
 781 GAAGTGATTC AAGTTAATCA TCAGACTGTG GTGGGGTGGC AGTTGAAAAA TTTGGTGAAT
 841 GCACTACGAG AGGACCCGAG TGGTGTTATC TTAACTTTGA AAAAGCGACC TCAGAGCATG
 901 CTTACCTCAG CACCAGCTTT ACTGAAAAAT ATGAGATGGA AGCCCCTTGC TCTGCAGCCT
 961 CTTATACCTA GAAGTCCCAC AAGCAGCGTT GCCACGCCTT CCAGCACCAT CAGTACACCC
1021 ACCAAAAGAG ACAGTTCTGC CCTCCAGGAT CTCTACATTC CCCCTCCTCC TGCAGAACCA
1081 TATATTCCCA GGGATGAAAA AGGAAACCTT CCTTGTGAAG ACCTCAGAGG ACATATGGTG
1141 GGCAAGCCAG TGCATAAGGG ATCTGAATCA CCAAATTCAT TTCTGGATCA GGAATATCGA
1201 AAGAGATTTA ATATTGTCGA AGAAGATACT GTCTTATATT GCTATGAATA TGAAAAAGGA
1261 AGATCAAGTA GTCAAGGAAG ACGAGAAAGC ACCCCAACTT ATGGCAAGCT ACGACCTATA
1321 TCTATGCCAG TGGAATATAA TTGGGTGGGG GACTATGAAG ATCCAAATAA GATGAAGAGA
1381 GATAGTAGAA GAGAAAACTC TCTACTTCGG TATATGAGCA ATGAAAAGAT TGCTCAAGAA
1441 GAATACATGT TTCAGAGAAA CAGCAAAAAG GACACAGGGA AGAAGTCAAA AAAGAAGGGT
1501 GATAAGAGTA ATAGCCCAAC TCACTATTCA TTGCTACCTA GTTTACAAAT GGATGCACTG
1561 AGACAAGACA TCATGGGCAC TCCTGTGCCA GAGACCACAC TATACCATAC ATTTCAGCAG
1621 TCCTCACTGC AGCACAAATC AAAGAAGAAA AACAAAGGTC CTATAGCAGG CAAGAGCAAA
1681 AGACGAATTT CTTGCAAAGA TCTTGGCCGT GGTGACTGTG AGGGCTGGCT TTGGAAAAAG
1741 AAAGATGCGA AGAGTTACTT TTCACAGAAA TGGAAAAAAT ATTGGTTTGT CCTAAAGGAT
1801 GCATCCCTTT ATTGGTATAT TAATGAGGAG GATGAAAAAG CAGAAGGATT CATTAGCCTG
1861 CCTGAATTTA AAATTGATAG AGCCAGTGAA TGCCGCAAAA AATATGCATT CAAAGCCTGT
1921 CATCCTAAAA TCAAAAGCTT TTATTTTGCT GCTGAACATC TTGATGATAT GAACAGGTGG
1981 CTTAACAGAA TTAATATGCT GACTGCAGGA TATGCAGAAA GAGAGAGGAT TAAGCAGGAA
2041 CAAGATTACT GGAGTGAGAG TGACAAGGAA GAAGCAGATA CTCCATCAAC ACCAAAACAA
2101 GATAGCCCTC CACCCCCATA TGATACATAC CCACGACCTC CCTCGATGAG TTGCGCCAGT
2161 CCTTATGTGG AAGCAAAACA TAGCCGACTT TCCTCCACGG AGACTTCTCA GTCTCAGTCT
2221 TCTCATGAGG AGTTTCGCCA GGAAGTAACT GGGAGCAGTG CAGTGTCTCC CATTCGCAAG
2281 ACAGCCAGTC AGCGCCGCTC CTGGCAGGAT TTAATTGAGA CGCCACTGAC AAGTTCAGGC
2341 TTACACTATC TTCAGACTCT GCCCCTGGAG GATTCTGTCT TCTCTGACTC CGCGGCCATC
2401 TCCCCAGAGC ACAGGCGGCA GTCTACCCTG CCAACTCAGA AATGCCACCT GCAGGATCAC
2461 TATGGGCCAT ACCCCTTAGC TGAGAGTGAG AGGATGCAAG TGCTAAATGG AAATGGGGGC
2521 AAGCCTCGAA GTTTTACTCT GCCTCGAGAT AGCGGGTTCA ACCATTGCTG TCTGAATGCT
2581 CCAGTTAGTG CCTGTGACCC ACAGGATGAC GTGCAACCCC CAGAGGTGGA GGAAGAGGAG
2641 GAGGAGGAGG AGGAGGAAGG GGAGGCAGCA GGGGAAAACA TAGGAGAAAA AAGTGAAAGC
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2701 AGAGAAGAAA AGTTAGGAGA CTCATTGCAA GATTTATACA GGGCACTGGA GCAGGCCAGT
2761 CTGTCACCAC TAGGAGAACA TCGTATTTCA ACCAAGATGG AATACAAGCT ATCATTTATA
2821 AAAAGATGTA ATGATCCTGT AATGAATGAA AAACTACACC GGCTGAGAAT TCTCAAAAGC
2881 ACTTTAAAGG CCAGAGAAGG GGAAGTAGCC ATTATCGATA AAGTCCTAGA CAATCCAGAC
2941 TTGACATCTA AAGAATTCCA ACAATGGAAG CAGATGTACC TCGACCTTTT CTTGGATATC
3001 TGTCAAAATA CCACCTCAAA TGACCCACTG AGTATTTCTT CTGAAGTAGA TGTAATCACT
3061 TCCTCTCTAG CACACACTCA TTCATACATT GAAACGCATG TCTAA

Figure 10: SEQ ID NO. 4: nucleotide sequence of human MAGUIN-2 coding sequence

Length: 2683 bp

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   1  ATGGCTCTGA TAATGGAACC GGTGAGCAAA TGGTCTCCGA GTCAAGTAGT
  51  GGACTGGATG AAAGGTCTTG ATGACTGTTT GCAGCAGTAT ATTAAGAACT
 101  TTGAGAGGGA GAAGATCAGT GGGGACCAGC TGCTGCGCAT TACACATCAG
 151  GAGCTAGAAG ATCTGGGGGT CAGCCGCATT GGCCATCAGG AACTGATCTT
 201  GGAAGCAGTT GACCTTCTGT GTGCATTGAA TTATGGCTTG GAAACAGAAA
 251  ATCTAAAAAC CCTTTCTCAC AAGTTGAATG CATCTGCCAA AAATCTGCAG
 301  AATTTTATAA CAGGAAGGAG AAGGAGTGGC CATTATGATG GGAGGACCAG
 351  CCGAAAATTG CCAAACGACT TTCTGACCTC AGTTGTGGAT CTGATTGGAG
 401  CAGCCAAGAG TCTGCTTGCC TGGTTGGACA GGTCACCATT TGCTGCTGTG
 451  ACAGACTATT CAGTTACAAG AAATAATGTC ATACAACTCT GCCTGGAGTT
 501  AACAACAATT GTGCAACAGG ATTGTACTGT ATATGAAACA GAGAATAAAA
 551  TTCTTCACGT GTGTAAAACT CTTTCTGGAG TCTGTGACCA CATCATATCC
 601  CTGTCGTCAG ATCCTCTGGT TTCACAGTCT GCTCACCTGG AAGTGATTCA
 651  GCTGGCAAAC ATTAAACCAA GCGAAGGGCT GGGTATGTAT ATTAAATCTA
 701  CATATGATGG CCTCCATGTA ATTACTGGAA CCACAGAAAA TTCACCTGCA
 751  GATCGGTGCA AGAAAATCCA TGCTGGCGAT GAAGTGATTC AAGTTAATCA
 801  TCAGACTGTG GTGGGGTGGC AGTTGAAAAA TTTGGTGAAT GCACTACGAG
 851  AGGACCCGAG TGGTGTTATC TTAACTTTGA AAAAGCGACC TCAGAGCATG
 901  CTTACCTCAG CACCAGCTTT ACTGAAAAAT ATGAGATGGA AGCCCCTTGC
 951  TCTGCAGCCT CTTATACCTA GAAGTCCCAC AAGCAGCGTT GCCACGCCTT
1001  CCAGCACCAT CAGTACACCC ACCAAAAGAG ACAGTTCTGC CCTCCAGGAT
1051  CTCTACATTC CCCCTCCTCC TGCAGAACCA TATATTCCCA GGGATGAAAA
1101  AGGAAACCTT CCTTGTGAAG ACCTCAGAGG ACATATGGTG GGCAAGCCAG
1151  TGCATAAGGG ATCTGAATCA CCAAATTCAT TTCTGGATCA GGAATATCGA
1201  AAGAGATTTA ATATTGTCGA AGAAGATACT GTCTTATATT GCTATGAATA
1251  TGAAAAAGGA AGATCAAGTA GTCAAGGAAG ACGAGAAAGC ACCCCAACTT
1301  ATGGCAAGCT ACGACCTATA TCTATGCCAG TGGAATATAA TTGGGTGGGG
1351  GACTATGAAG ATCCAAATAA GATGAAGAGA GATAGTAGAA GAGAAAACTC
1401  TCTACTTCGG TATATGAGCA ATGAAAAGAT TGCTCAAGAA GAATACATGT
1451  TTCAGAGAAA CAGCAAAAAG GACACAGGGA AGAAGTCAAA AAAGAAGGGT
1501  GATAAGAGTA ATAGCCCAAC TCACTATTCA TTGCTACCTA GTTTACAAAT
1551  GGATGCACTG AGACAAGACA TCATGGGCAC TCCTGTGCCA GAGACCACAC
1601  TATACCATAC ATTTCAGCAG TCCTCACTGC AGCACAAATC AAAGAAGAAA
1651  AACAAAGGTC CTATAGCAGG CAAGAGCAAA AGACGAATTT CTTGCAAAGA
1701  TCTTGGCCGT GGTGACTGTG AGGGCTGGCT TTGGAAAAAG AAAGATGCGA
1751  AGAGTTACTT TTCACAGAAA TGGAAAAAAT ATTGGTTTGT CCTAAAGGAT
1801  GCATCCCTTT ATTGGTATAT TAATGAGGAG GATGAAAAAG CAGAAGGATT
1851  CATTAGCCTG CCTGAATTTA AAATTGATAG AGCCAGTGAA TGCCGCAAAA
1901  AATATGCATT CAAAGCCTGT CATCCTAAAA TCAAAAGCTT TTATTTTGCT
1951  GCTGAACATC TTGATGATAT GAACAGGTGG CTTAACAGAA TTAATATGCT
2001  GACTGCAGGA TATGCAGAAA GAGAGAGGAT TAAGCAGGAA CAAGATTACT
2051  GGAGTGAGAG TGACAAGGAA GAAGCAGATA CTCCATCAAC ACCAAAACAA
2101  GATAGCCCTC CACCCCCATA TGATACATAC CCACGACCTC CCTCGATGAG
2151  TTGCGCCAGT CCTTATGTGG AAGCAAAACA TAGCCGACTT TCCTCCACGG
2201  AGACTTCTCA GTCTCAGTCT TCTCATGAGG AGTTTCGCCA GGAAGTAACT
2251  GGGAGCAGTG CAGTGTCTCC CATTCGCAAG ACAGCCAGTC AGCGCCGCTC
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2301  CTGGCAGG[redacted]TTAATTGAGA CGCCACTGAC AAGTTCAGGC[redacted]ACACTATC
2351  TTCAGACTCT GCCCCTGGAG GATTCTGTCT TCTCTGACTC CGCGGCCATC
2401  TCCCCAGAGC ACAGGCGGCA GTCTACCCTG CCAACTCAGA AATGCCACCT
2451  GCAGGATCAC TATGGGCCAT ACCCCTTAGC TGAGAGTGAG AGGATGCAAG
2501  TGCTAAATGG AAATGGGGGC AAGCCTCGAA GTTTTACTCT GCCTCGAGAT
2551  AGCGGGTTCA ACCATTGCTG TCTGAaTGCT CCAGTTAGTG CCTGTGACCC
2601  ACAGGATGAC GTGCAACCCC CAGAGGTGGA GGAAGAGGAG GAGGAGGAGG
2651  AGGAGGAAGG GGAGGCAGCA GGGGAAAACA TAG
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Figure 11: SEQ ID NO. 5: nucleotide sequence of human MAGUIN-1 cDNA

Length: 5749 bp

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   1 CGGGCAGCTA GTCGTGCTCG GGGCTTCACT CCCGCGCGTG AGGCGAGCGG GCAAGTTGGC
  61 TGAGGGCGTG CGGCAGAGGC TGCTTCCCTC GGCGACGCGA CCCCTCAGCA ACTCAAGCTA
 121 TGAACTGAAG CTCCCTAGGG ACGGAGACCG GAGCGGAGCG GCGGAGGCAG CAGCAGCAGC
 181 AGCAGCAGCA GCAGCAGCAG CAGCCGCCGC CGCCGCCGCC TTAGCGGGAA CTGAGCAGAC
 241 CCGGCGCGGA GCCACGACTC CTGCACGTTT ACCTCCCTGT CGCCGTTCCT GCCGGCGGTT
 301 GGCTAAAAGA CGTTACAGCC GCGAGACCCG ACACACAAAA GCCGCTTTCT CCGCGCCGCC
 361 CGCCCAGGGA GGCTGCGGCC AGCAAGGGAC CCCACCTGAG AGCAGCTCGG GCTGCTGAGT
 421 TCGTTTTGTG TCTGAGCTCT GCGCTCTGCA CGGAACCGAC CCCGTACCCA TGGCTCTGAT
 481 AATGGAACCG GTGAGCAAAT GGTCTCCGAG TCAAGTAGTG GACTGGATGA AAGGTCTTGA
 541 TGACTGTTTG CAGCAGTATA TTAAGAACTT TGAGAGGGAG AAGATCAGTG GGGACCAGCT
 601 GCTGCGCATT ACACATCAGG AGCTAGAAGA TCTGGGGGTC AGCCGCATTG GCCATCAGGA
 661 ACTGATCTTG GAAGCAGTTG ACCTTCTGTG TGCATTGAAT TATGGCTTGG AAACAGAAAA
 721 TCTAAAAACC CTTTCTCACA AGTTGAATGC ATCTGCCAAA AATCTGCAGA ATTTTATAAC
 781 AGGAAGGAGA AGGAGTGGCC ATTATGATGG GAGGACCAGC CGAAAATTGC CAAACGACTT
 841 TCTGACCTCA GTTGTGGATC TGATTGGAGC AGCCAAGAGT CTGCTTGCCT GGTTGGACAG
 901 GTCACCATTT GCTGCTGTGA CAGACTATTC AGTTACAAGA AATAATGTCA TACAACTCTG
 961 CCTGGAGTTA ACAACAATTG TGCAACAGGA TTGTACTGTA TATGAAACAG AGAATAAAAT
1021 TCTTCACGTG TGTAAAACTC TTTCTGGAGT CTGTGACCAC ATCATATCCC TGTCGTCAGA
1081 TCCTCTGGTT TCACAGTCTG CTCACCTGGA AGTGATTCAA CTGGCAAACA TTAAACCAAG
1141 CGAAGGGCTG GGTATGTATA TTAAATCTAC ATATGATGGC CTCCATGTAA TTACTGGAAC
1201 CACAGAAAAT TCACCTGCAG ATCGGTGCAA GAAAATCCAT GCTGGCGATG AAGTGATTCA
1261 AGTTAATCAT CAGACTGTGG TGGGGTGGCA GTTGAAAAAT TTGGTGAATG CACTACGAGA
1321 GGACCCGAGT GGTGTTATCT TAACTTTGAA AAAGCGACCT CAGAGCATGC TTACCTCAGC
1381 ACCAGCTTTA CTGAAAAATA TGAGATGGAA GCCCCTTGCT CTGCAGCCTC TTATACCTAG
1441 AAGTCCCACA AGCAGCGTTG CCACGCCTTC CAGCACCATC AGTACACCCA CCAAAAGAGA
1501 CAGTTCTGCC CTCCAGGATC TCTACATTCC CCCTCCTCCT GCAGAACCAT ATATTCCCAG
1561 GGATGAAAAA GGAAACCTTC CTTGTGAAGA CCTCAGAGGA CATATGGTGG GCAAGCCAGT
1621 GCATAAGGGA TCTGAATCAC CAAATTCATT TCTGGATCAG GAATATCGAA AGAGATTTAA
1681 TATTGTCGAA GAAGATACTG TCTTATATTG CTATGAATAT GAAAAAGGAA GATCAAGTAG
1741 TCAAGGAAGA CGAGAAAGCA CCCCAACTTA TGGCAAGCTA CGACCTATAT CTATGCCAGT
1801 GGAATATAAT TGGGTGGGGG ACTATGAAGA TCCAAATAAG ATGAAGAGAG ATAGTAGAAG
1861 AGAAAACTCT CTACTTCGGT ATATGAGCAA TGAAAAGATT GCTCAAGAAG AATACATGTT
1921 TCAGAGAAAC AGCAAAAAGG ACACAGGGAA GAAGTCAAAA AAGAAGGGTG ATAAGAGTAA
1981 TAGCCCAACT CACTATTCAT TGCTACCTAG TTTACAAATG GATGCACTGA GACAAGACAT
2041 CATGGGCACT CCTGTGCCAG AGACCACACT ATACCATACA TTTCAGCAGT CCTCACTGCA
2101 GCACAAATCA AAGAAGAAAA ACAAAGGTCC TATAGCAGGC AAGAGCAAAA GACGAATTTC
2161 TTGCAAAGAT CTTGGCCGTG GTGACTGTGA GGGCTGGCTT TGGAAAAAGA AAGATGCGAA
2221 GAGTTACTTT TCACAGAAAT GGAAAAAATA TTGGTTTGTC CTAAAGGATG CATCCCTTTA
2281 TTGGTATATT AATGAGGAGG ATGAAAAAGC AGAAGGATTC ATTAGCCTGC CTGAATTTAA
2341 AATTGATAGA GCCAGTGAAT GCCGCAAAAA ATATGCATTC AAAGCCTGTC ATCCTAAAAT
2401 CAAAAGCTTT TATTTTGCTG CTGAACATCT TGATGATATG AACAGGTGGC TTAACAGAAT
2461 TAATATGCTG ACTGCAGGAT ATGCAGAAAG AGAGAGGATT AAGCAGGAAC AAGATTACTG
2521 GAGTGAGAGT GACAAGGAAG AAGCAGATAC TCCATCAACA CCAAAACAAG ATAGCCCTCC
2581 ACCCCCATAT GATACATACC CACGACCTCC CTCGATGAGT TGCGCCAGTC CTTATGTGGA
2641 AGCAAAACAT AGCCGACTTT CCTCCACGGA GACTTCTCAG TCTCAGTCTT CTCATGAGGA
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2701 GTTTCGCCAG GAAGTAACTG GGAGCAGTGC AGTGTCTCCC ATTCGCAAGA CAGCCAGTCA
2761 GCGCCGCTCC TGGCAGGATT TAATTGAGAC GCCACTGACA AGTTCAGGCT TACACTATCT
2821 TCAGACTCTG CCCCTGGAGG ATTCTGTCTT CTCTGACTCC GCGGCCATCT CCCCAGAGCA
2881 CAGGCGGCAG TCTACCCTGC CAACTCAGAA ATGCCACCTG CAGGATCACT ATGGGCCATA
2941 CCCCTTAGCT GAGAGTGAGA GGATGCAAGT GCTAAATGGA AATGGGGGCA AGCCTCGAAG
3001 TTTTACTCTG CCTCGAGATA GCGGGTTCAA CCATTGCTGT CTGAATGCTC CAGTTAGTGC
3061 CTGTGACCCA CAGGATGACG TGCAACCCCC AGAGGTGGAG GAAGAGGAGG AGGAGGAGGA
3121 GGAGGAAGGG GAGGCAGCAG GGGAAAACAT AGGAGAAAAA AGTGAAAGCA GAGAAGAAAA
3181 GTTAGGAGAC TCATTGCAAG ATTTATACAG GGCACTGGAG CAGGCCAGTC TGTCACCACT
3241 AGGAGAACAT CGTATTTCAA CCAAGATGGA ATACAAGCTA TCATTTATAA AAAGATGTAA
3301 TGATCCTGTA ATGAATGAAA AACTACACCG GCTGAGAATT CTCAAAAGCA CTTTAAAGGC
3361 CAGAGAAGGG GAAGTAGCCA TTATCGATAA AGTCCTAGAC AATCCAGACT TGACATCTAA
3421 AGAATTCCAA CAATGGAAGC AGATGTACCT CGACCTTTTC TTGGATATCT GTCAAAATAC
3481 CACCTCAAAT GACCCACTGA GTATTTCTTC TGAAGTAGAT GTAATCACTT CCTCTCTAGC
3541 ACACACTCAT TCATACATTG AAACGCATGT CTAAATGTAT TCTGCCTTCA GACCATCTAG
3601 TACCTGCTGG TACTCTGAAC AAGTATATAA GGTAGTTTTT ATATCAATGT GTGGAACACT
3661 TGACAAGCTA TACTTTAATG TTACCAAACT ATATGAAACA AACCATATAT GGTCACAATA
3721 CCACTATCTT TAATGAGCAT TTGTATATTT TATATGCAAC AGTGCTCAGC TTATGTTTAC
3781 CATGTGCAAA ATCAACTGTC TTTAATGACT TAAAATTAAC TTTTGCAAAC AATTCTAAAT
3841 ACAGGTGGTC TTCAAGTAGT AAAACCACAA AAGGCAGTTT TCTATCTATG GTCATCTTTT
3901 CTCCCTTTAA GTTAATTTTA TATAAACAAG ACTTCAAAAG TAAATCACAT TTTTTCAGGT
3961 GCAGACATCC TTGTGGGTGG GAAAGAATTT AAACCTTTTT TATATTTATT AAAATGTTCT
4021 AAGAATTTTC TTAAACATTG CACAAAGTTT AATGCTGTAG TTTTATTTTT GTGAAATGTA
4081 GATGCGCATA CAAGAGCTAA GCAAAATAGA AGAGCATCGA CATAAGAAAA GTTCAGGTAT
4141 CTAATATTCG TCTTAATAGT CTATTAACTT GTGAAAGCTA AGTTAATGGA AATATTATTC
4201 CAAATCTATG AGAACACTTG GTGTATCAGG GCAAAGCTTT GTAAGATGTT TTTGTAACTA
4261 AGACCAAAAT TGAAGATAGA GCTGCTTTAT TTTCTTGGTT TAAATCTTCC TTTATTTTTG
4321 TAGTGATGAG ATGCTGATTG TGTACAGAAG AATTTGAGAG GGGATTTTTA AAAACTGACT
4381 TAACACACCC AGAAAGGCAG CTAACAGCTA TATATATATA TAAATTTCAG CCCAAACTCA
4441 TGTTTTTAAA CTCCAACTCT TAAAAGACAA CAAGGTATAA ACTGAAATGA ATCAACTTTC
4501 CACTTAGTTT CCAATTTTCC CCTAGTCCAC TAATTAAACT TAGGTAATTA TACTTCAGGT
4561 AGGGAAGTAC AATATGTTTA GTTTCAGGCT GATGTGTGTT ATAAAAAACA ACACTGAAAA
4621 ATAAAAATGT ACTTCCCTTC TAAGGAGCAA GCAGGTGATG GTCATTCAAA GAGATGTCAC
4681 ATTGAATTAT GAGAGAAACA ATTTAGAGGT TTTTTTCCTG GCTTCATGAA TTGTTCTATA
4741 GAGTGGATGA AGTCTAAGGA AAAGTCCTCT TCATATATTT CCATTTATAA GCGTCTTGTT
4801 TTTGAAAGTG ATCACAGCAT GAAAATGACT GTGCTGCTTT TTAGTGTCTG GCTGCATAAT
4861 GTACAAGTCA CAATTTGCTG TTTTTTTCAG GAGGAGAAAG GGAACCTCCT TTACTATTCT
4921 ATATCCTAAA ATCTACTTCT AATCAGCTTT ATACTGTTGC CTGTACAGCT CAGTGAATGT
4981 ACTTTCATCT TTAAGAGTTC AGATATATGC CAGTGAATAT TTTTGCTGTA GAGGAGAAAG
5041 TAAAAACTCC ACAGCGGGGA TCTTTTTCTT TGCTTTTGAA ACCACCATTG AATCACTATC
5101 GTTTTGCAGA CTTTGCACAA CTGTACAGGA GAGTGGCCTT TCTACAGCAC ATTTTCAGTA
5161 ATCCTATATT TAGTCAAAAT GGATGAGAAA TCATGTATTA ATGTTTGTAT GGAATTTTGG
5221 GTCCAGTGTA ATATTTTTAT CATTTAAAAA GAACTCTATT TGTAAAAACA TTTATTTACT
5281 GCATGGATAT TGACGCACAT TAAATTTGTG GGATTTTGTA TATGTAAAAA AAAAAAAAAA
5341 AAAAAAAAAC AAAAAACCTC TTGTCCTAAA ATGAAGTGTG CTTGTTAACA GGTGTTTAGA
5401 CTTATTGATG TTTACTAGAC CAAATGTGTA TGTTCACTTA AAAATATATG TACCTGATGG
5461 ATGTGTCATG TTTACAGTGG CCAGGTTGTG GCCTGTAAAC AGCAAGCAGT TGACGGGAAG
5521 ACTAGCTCTG TTGCTACTAA GCAGCTTTTA CTTTTGTAAA GTCAGCTCTG TTGTTTTAAA
5581 TGGTAAAAAT TAAACTAATG AATTTGACAA GACTCGTGGC TAGCCTAGCA TGAAAGAGAC
5641 CTTTTAACAC TATATAATAT CTGTACATTT TATTGCATTC GTTTCAAATC TAGGAGAGAG
5701 GCAGCACTGT AAACTGAAGT CAAATAAATT CAGCTCTTAA TGAATCCTT
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Figure 12: SEQ ID NO. 6: nucleotide sequence of human MAGUIN-2 cDNA

Length: 4350 bp

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   1 GTGCTCGGGG CTTCACTCCC GCGCGTGAGG CGAGCGGGCA AGTTGGCTGA
  51 GGGCGTGCGG CAGAGGCTGC TTCCCTCGGC GACGCGACCC CTCAGCAACT
 101 CAAGCTATGA ACTGAAGCTC CCTAGGGACG GAGACCGGAG CGGAGCGGCG
 151 GAGGCAGCAG CAGCAGCAGC AGCAGCAGCA GCAGCAGCAG CCGCCGCCGC
 201 CGCCGCCTTA GCGGGAACTG AGCAGACCCG GCGCGGAGCC ACGACTCCTG
 251 CACGTTTACC TCCCTGTCGC CGTTCCTGCC GGCGGTTGGC TAAAAGACGT
 301 TACAGCCGCG AGACCCGACA CACAAAAGCC GCTTTCTCCG CGCCGCCCGC
 351 CCAGGGAGGC TGCGGCCAGC AAGGGACCCC ACCTGAGAGC AGCTCGGGCT
 401 GCTGAGTTCG TTTTGTGTCT GAGCTCTGCG CTCTGCACGG AACCGACCCC
 451 GTACCCATGG CTCTGATAAT GGAACCGGTG AGCAAATGGT CTCCGAGTCA
 501 AGTAGTGGAC TGGATGAAAG GTCTTGATGA CTGTTTGCAG CAGTATATTA
 551 AGAACTTTGA GAGGGAGAAG ATCAGTGGGG ACCAGCTGCT GCGCATTACA
 601 CATCAGGAGC TAGAAGATCT GGGGGTCAGC CGCATTGGCC ATCAGGAACT
 651 GATCTTGGAA GCAGTTGACC TTCTGTGTGC ATTGAATTAT GGCTTGGAAA
 701 CAGAAAATCT AAAAACCCTT TCTCACAAGT TGAATGCATC TGCCAAAAAT
 751 CTGCAGAATT TTATAACAGG AAGGAGAAGG AGTGGCCATT ATGATGGGAG
 801 GACCAGCCGA AAATTGCCAA ACGACTTTCT GACCTCAGTT GTGGATCTGA
 851 TTGGAGCAGC CAAGAGTCTG CTTGCCTGGT TGGACAGGTC ACCATTTGCT
 901 GCTGTGACAG ACTATTCAGT TACAAGAAAT AATGTCATAC AACTCTGCCT
 951 GGAGTTAACA ACAATTGTGC AACAGGATTG TACTGTATAT GAAACAGAGA
1001 ATAAAATTCT TCACGTGTGT AAAACTCTTT CTGGAGTCTG TGACCACATC
1051 ATATCCCTGT CGTCAGATCC TCTGGTTTCA CAGTCTGCTC ACCTGGAAGT
1101 GATTCAGCTG GCAAACATTA AACCAAGCGA AGGGCTGGGT ATGTATATTA
1151 AATCTACATA TGATGGCCTC CATGTAATTA CTGGAACCAC AGAAAATTCA
1201 CCTGCAGATC GGTGCAAGAA AATCCATGCT GGCGATGAAG TGATTCAAGT
1251 TAATCATCAG ACTGTGGTGG GGTGGCAGTT GAAAAATTTG GTGAATGCAC
1301 TACGAGAGGA CCCGAGTGGT GTTATCTTAA CTTTGAAAAA GCGACCTCAG
1351 AGCATGCTTA CCTCAGCACC AGCTTTACTG AAAAATATGA GATGGAAGCC
1401 CCTTGCTCTG CAGCCTCTTA TACCTAGAAG TCCCACAAGC AGCGTTGCCA
1451 CGCCTTCCAG CACCATCAGT ACACCCACCA AAAGAGACAG TTCTGCCCTC
1501 CAGGATCTCT ACATTCCCCC TCCTCCTGCA GAACCATATA TTCCCAGGGA
1551 TGAAAAAGGA AACCTTCCTT GTGAAGACCT CAGAGGACAT ATGGTGGGCA
1601 AGCCAGTGCA TAAGGGATCT GAATCACCAA ATTCATTTCT GGATCAGGAA
1651 TATCGAAAGA GATTTAATAT TGTCGAAGAA GATACTGTCT TATATTGCTA
1701 TGAATATGAA AAAGGAAGAT CAAGTAGTCA AGGAAGACGA GAAAGCACCC
1751 CAACTTATGG CAAGCTACGA CCTATATCTA TGCCAGTGGA ATATAATTGG
1801 GTGGGGGACT ATGAAGATCC AAATAAGATG AAGAGAGATA GTAGAAGAGA
1851 AAACTCTCTA CTTCGGTATA TGAGCAATGA AAAGATTGCT CAAGAAGAAT
1901 ACATGTTTCA GAGAAACAGC AAAAAGGACA CAGGGAAGAA GTCAAAAAAG
1951 AAGGGTGATA AGAGTAATAG CCCAACTCAC TATTCATTGC TACCTAGTTT
2001 ACAAATGGAT GCACTGAGAC AAGACATCAT GGGCACTCCT GTGCCAGAGA
2051 CCACACTATA CCATACATTT CAGCAGTCCT CACTGCAGCA CAAATCAAAG
2101 AAGAAAAACA AAGGTCCTAT AGCAGGCAAG AGCAAAAGAC GAATTTCTTG
2151 CAAAGATCTT GGCCGTGGTG ACTGTGAGGG CTGGCTTTGG AAAAAGAAAG
2201 ATGCGAAGAG TTACTTTTCA CAGAAATGGA AAAAATATTG GTTTGTCCTA
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2251  AAGGATGCAT CCCTTTATTG GTATATTAAT GAGGAGGATG AAAAAGCAGA
2301  AGGATTCATT AGCCTGCCTG AATTTAAAAT TGATAGAGCC AGTGAATGCC
2351  GCAAAAAATA TGCATTCAAA GCCTGTCATC CTAAAATCAA AAGCTTTTAT
2401  TTTGCTGCTG AACATCTTGA TGATATGAAC AGGTGGCTTA ACAGAATTAA
2451  TATGCTGACT GCAGGATATG CAGAAAGAGA GAGGATTAAG CAGGAACAAG
2501  ATTACTGGAG TGAGAGTGAC AAGGAAGAAG CAGATACTCC ATCAACACCA
2551  AAACAAGATA GCCCTCCACC CCCATATGAT ACATACCCAC GACCTCCCTC
2601  GATGAGTTGC GCCAGTCCTT ATGTGGAAGC AAAACATAGC CGACTTTCCT
2651  CCACGGAGAC TTCTCAGTCT CAGTCTTCTC ATGAGGAGTT TCGCCAGGAA
2701  GTAACTGGGA GCAGTGCAGT GTCTCCCATT CGCAAGACAG CCAGTCAGCG
2751  CCGCTCCTGG CAGGATTTAA TTGAGACGCC ACTGACAAGT TCAGGCTTAC
2801  ACTATCTTCA GACTCTGCCC CTGGAGGATT CTGTCTTCTC TGACTCCGCG
2851  GCCATCTCCC CAGAGCACAG GCGGCAGTCT ACCCTGCCAA CTCAGAAATG
2901  CCACCTGCAG GATCACTATG GGCCATACCC CTTAGCTGAG AGTGAGAGGA
2951  TGCAAGTGCT AAATGGAAAT GGGGGCAAGC CTCGAAGTTT TACTCTGCCT
3001  CGAGATAGCG GGTTCAACCA TTGCTGTCTG AaTGCTCCAG TTAGTGCCTG
3051  TGACCCACAG GATGACGTGC AACCCCCAGA GGTGGAGGAA GAGGAGGAGG
3101  AGGAGGAGGA GGAAGGGGAG GCAGCAGGGG AAAACATAGG AGAAAAAAGC
3151  TAATACACTG CGAGAGTTGG TAGAACCTCT CCATGCCAAA TCGGATCCAC
3201  TTCTGTTGGC ACTCAACCCA TTGGACTCAC AGATTGATAA GCTAATGTTT
3251  AGAGAATTTA GATCGGAGAG AGTCGGTACG GCGCAGACTC AACATCAACC
3301  TCTTGCAAGC AACTAAAATG GCCTCGTCCT TGCTGTTTAT AACAGAAAAC
3351  AGACTTGTAA AAAGCTTAGA TCATCAAGTG TTTTGGATTG GGGGCCTCCC
3401  AAAGGGATAT AAGAGGGGCA GGCCACTCTT AAGAAGAATG CGAGCTTTCT
3451  ACATTGGGAC TAGCATAAGA TCAAAGCCAA TCAAGATGGA GCACAGTAAC
3501  AGAAAACTGC GGTTTCTGTG GGAGAACAGA AGGGGAAAGG GTCTTAACTG
3551  GGAAAGGGCT CTGTGTGGTA ACACCTCAGT TGTGTTCTCC TGACACCAGG
3601  AAAAGAGAGG GATCAGCTTC AATAACTAGA AAATTCTGGC TGTTTAATGG
3651  ACTCTTTGGT GGCCTCTTTA AGGCAAAGCA GAGAAAGCAA ATTATGTATT
3701  AAGTGTATTT TGCATTTTTA AAACTTGACG TGCTGTATTG TACTAAATTA
3751  AGTGTAATCT ATTAAGGCAA GGTATACACA ATTTGCTTTG AAACTTACTA
3801  TGTTTATTCT ATTATAAAGT GTATTCAGGT GCAACACAGA GACTGCTTTC
3851  GGTGACATTA ATGAAGAAAA TTTCTCATGC CAGGCTTTAT TATAGAATCT
3901  TCAGCTAAAA TCCTAACTTT CTCCTTATTT CTTGGCACTT GTATACAAGT
3951  GGTGTTGCCT CTTAGGGCAG GCATGAGCTA TTCTTTTCTG TAAAATATTT
4001  TGAATCTATA GGCTGTGGGT TTCATTTTTG AAAAGTATTT TGTCTGGATG
4051  TCTTTCAAAC TAGCTTCAGA TATTATTTAA TACTATGTAA CTGGGTCCCC
4101  TATGGCTCAA TCAATATTGC TTATTTTTCT TCTGTAGTGG ATGTGAAATT
4151  TCCTTTAGTT GGATAAGATA CACTGTAATA ATTTTAATGC TAATTAATGA
4201  TATTTCATAC TGTGCAATGA ACAGATAATT TAACACTGTA TTTTGAAATG
4251  TTTTTTTCTT CCTGTCACCG CAGTGTGTGG TATTGCATAA TGTGAATACC
4301  TGTAAAAATA TAAATTACTT AAAAATAAAA ATATGACCAA TTGGTATCAG
```

Figure 13: SEQ ID NO. 7

Length: 50 bp

1 GGAGAGAGGCAGCACTGTAAACTGAAGTCAAATAAATTCAGCTCTTAATG

Figure 14: Alignment of SEQ ID NO. 7 with human MAGUIN-1 cDNA

Length: 50 bp

```
    1 GGAGAGAGGCAGCACTGTAAACTGAAGTCAAATAAATTCAGCTCTTAATG 50
      ||||||||||||||||||||||||||||||||||||||||||||||||||
 5693 GGAGAGAGGCAGCACTGTAAACTGAAGTCAAATAAATTCAGCTCTTAATG 5742
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Figure 15: Schematic alignment of SEQ ID NO. 5, SEQ ID NO. 6 and SEQ ID NO. 7 with Genome Database EST-cluster

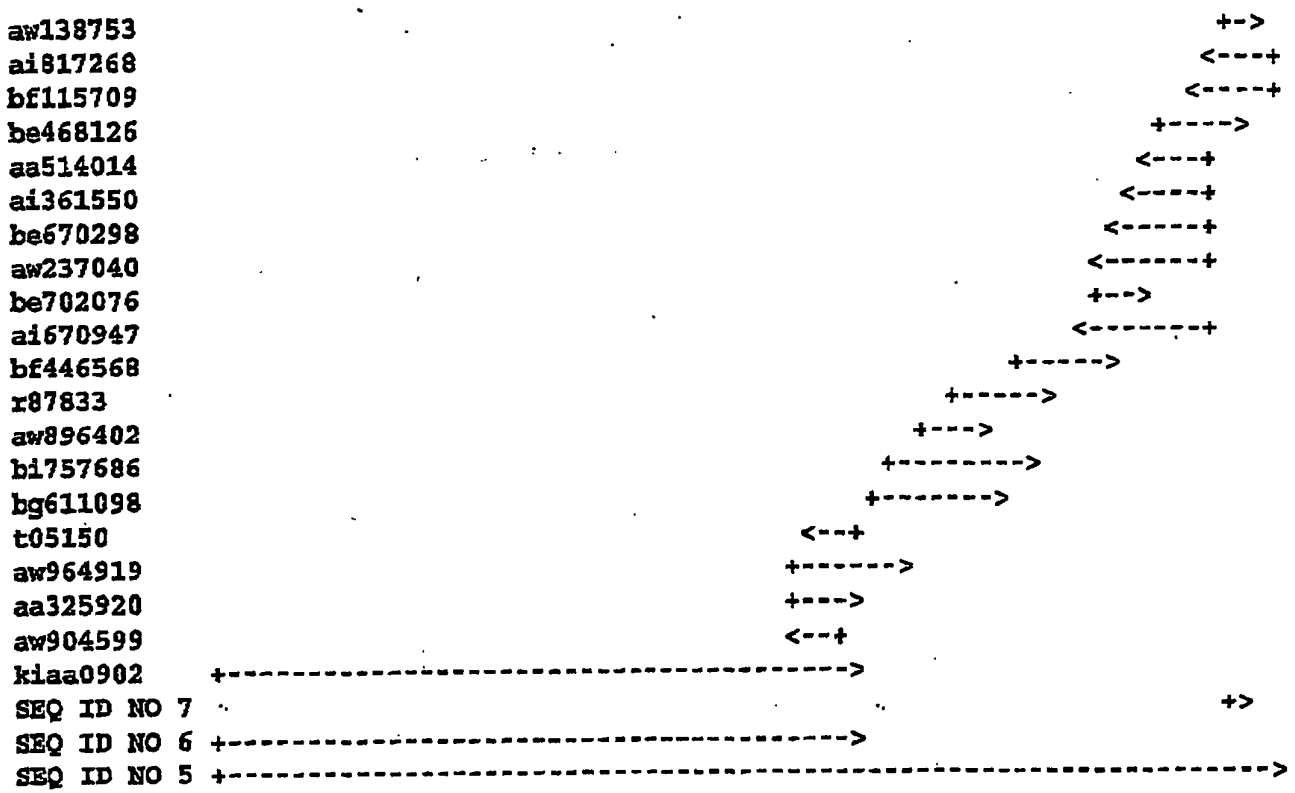

Table 1:

sample	Δ (fold) (frontal / temporal cortex)
patient 1	2.44
patient 2	2.78
patient 3	4.17
patient 4	2.17
patient 5	1.47
patient 6	1.42
patient 7	1.69
control 1	1.28
control 2	1.28
control 3	0.30
control 4	1.37
control 5	1.15

Table 2:

sample	Δ (fold) (frontal / temporal cortex)
patient 1	2.68
patient 2	2.72
patient 3	11.73
patient 4	2.44
patient 5	1.77
patient 6	3.43
patient 7	4.02
control 1	1.42
control 2	1.22
control 3	0.30
control 4	0.92
control 5	0.80

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